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Nutrient and phytochemical analyses of processed noni puree

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ABSTRACT

The recent approval of noni fruit puree as a novel food ingredient, as well as the growing popularity of this fruit in health beverages, will greatly increase its use in foodstuffs, and consequently, its consumption among the general population. As such, an understanding of the nutritional profile of processed noni fruit puree is important for food technologists, nutritionists, as well as consumers. Therefore, the proximate nutritional, vitamin, mineral, and amino acid contents were determined. The phytochemical properties were evaluated, as well as an assessment made on the safety and potential efficacy of the major phytochemicals present in the puree. Processed noni fruit puree is a potential dietary source of vitamin C, vitamin A, niacin, manganese, and selenium. Vitamin C is the major nutrient present, in terms of concentration. The major phytochemicals in the puree are iridoids, especially deacetylasperulosidic acid, which were present in higher concentrations than vitamin C. The iridoids in noni did not display any oral toxicity or genotoxicity, but did possess potential anti-genotoxic activity. These findings suggest that deacetylasperulosidic acid may play an important role in the biological activities of noni fruit juice that have been observed *in vitro*, *in vivo*, and in human clinical trials.
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1. Introduction

Morinda citrifolia, commonly known as noni, is a widely distributed tropical tree. It grows on the islands of the South Pacific, Southeast Asia, Central America, Indian subcontinent, and in the Caribbean. The fruit and leaves of this tree have a history of use both as food and for the promotion of health (Morton, 1992). The noni fruit juice industry has grown substantially in the past decade, especially since the approval of noni fruit juice from French Polynesia as a novel food ingredient by the Commission of the European Union (European Commission, 2003). Since this approval, other noni fruit juice products have been approved for sale within the E.U. under the simplified substantial equivalence procedure (European Communities, 1997). More than 40 commercial sources of noni fruit juice have been granted substantial equivalence. These sources are from a variety of nations which include French Polyensia, Fiji, Dominican Republic, Panama, Costa Rica, Samoa, U.S.A. (Hawaii), Tonga, Vanuatu, Cook Islands, Palau, Solomon Islands, and Nauru (DG SANCO, 2010).

Recent approval has also been given to extend the use of Polynesian noni fruit puree and fruit juice concentrate as novel food ingredients in a variety food categories (European Commission, 2010). These food categories include candy, cereal products, nutritional drink mixes, ice cream, yogurt, baked goods, jams and jellies, carbonated beverages, food supplements, spreads, fillings and icings, sauces, gravies, pickles and condiments.

The approval of noni fruit as a novel food ingredient will greatly increase the use of these ingredients in foodstuffs, and consequently, their consumption among the general population. As such, an understanding of the nutritional profile of processed noni fruit puree is important for food technologists, nutritionists, as well as consumers. Knowledge of the phytochemical profile of processed noni fruit is also important in understanding potential bioactivities, as well as in understanding the compounds responsible for health effects already demonstrated in human clinical trials. A few publications have provided some limited nutritional and phytochemical information on the composition of noni fruit. Proximate nutritional, fiber, sugar, partial amino acids, and some mineral analyses of juice pressed from raw noni fruits from Cambodia have been reported (Chunhieng et al., 2005). Vitamin C content, as well as that of five minerals, has been determined for wild noni fruit from northern Australia (Peerzada et al., 1990). Proximate nutritional, some minerals, vitamin A, and vitamin C contents of whole unprocessed noni fruits in Pohnpei have also been reported (Shovic & Whistler, 2001). Phytochemical investigations of raw noni fruits, and some commercial juices, have identified the presence of several different types of compounds (Basar & Westendorf, 2010; Potterat et al., 2007; Kamiya et al., 2005). But iridoids constitute the major phytochemical component of noni fruit (Deng et al., 2011), with a few other compounds, such as scopoletin, quercetin, and rutin occurring in significant, although much less, quantities (Deng, West, & Jensen, 2010). These previous analyses have been limited in the amount of nutrient data provided. Further, they have not been representative of commercially processed noni fruit puree, as processing conditions do alter the nutritional and phyto-chemical profiles of fruits and vegetables (Murcia et al., 2009;

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Rodrigues et al., 2009). Therefore, the current chemical analyses were performed to provide more complete and accurate nutritional data. Analyses of the major phytochemicals in noni fruit were also carried out to provide an important reference for quality control and identity testing of these raw materials.

As iridoids are present in significant quantities in noni fruit puree, genotoxicity and acute toxicity tests were performed to better understand their individual safety profiles. Noni fruit juice has also been shown to protect DNA against chemical mutagens *in vivo* and in a human clinical trial (Wang & Su, 2001; Wang, Peng, et al., 2009). Therefore, the anti-genotoxic activities of the iridoids were evaluated *in vitro* to investigate their potential roles in this reported DNA protection.

2. Materials and methods

2.1. Experimental materials

Noni fruits were harvested in French Polynesia and allowed to fully ripen. The fruit was then processed into a puree by mechanical removal of the seeds and skin via micro-mesh screen in a commercial fruit pulper, followed by pasteurization (87 °C for 3 s) at a good manufacturing certified fruit processing facility in Mataiea, Tahiti. The pasteurized puree was filled into aseptic containers, or totes containing 880 kg of noni fruit puree, and stored under refrigeration. For the chemical analyses in this study, samples were obtained from 10 different batches of puree.

For the acute oral toxicity test, an iridoid enriched fruit extract was prepared. This was done by removal of seeds and skin from the fruit flesh, followed by size reduction with a 0.65 mm sieve. An aqueous extract was prepared with the remaining fruit pulp, at ambient temperature, which was then freeze-dried, resulting in a total iridoid concentration of 1690 mg/100 g extract.

Freeze-dried noni fruit powder (36 g) was extracted with 1 L of methanol by percolation to produce 10 g of methanol extract. Following addition of water, the methanol extract was partitioned with ethyl acetate (150 mL, three times) to remove non-polar impurities. The aqueous extract was further partitioned with n-butanol (150 mL, three times) to yield 3 g n-butanol extract. The extract was subjected to flash column chromatography on silica gel, eluting with a stepwise dichloromethane: methanol (20:1 → 1.5:1) gradient solvent system to yield sixty-two primary fractions. Among these, the presence of two major compounds was indicated by a preliminary HPLC analysis. The iridoid containing fractions were combined and subject to further purification by using reverse phase preparative HPLC (Symmetry PrepTM C18 column, Waters Corp, Milford, Massachusetts, USA), eluting with an isocratic solvent system of acetonitrile: water (35:65, v:v) at a flow rate of 3 mL min^{-1}, resulting in the isolation of DAA and AA.

2.2. Chemical analyses

Proximate nutritional analyses of noni fruit puree were carried out to determine moisture, fat, protein, ash, and carbohydrate contents. Protein content was determined by the Kjeldahl method, Association of Official Analytical Chemists (AOAC) Method 979.09 (AOAC, 2000 a), with a Kjeltec System 1002 distilling unit (Foss Tecator, Höganäs, Sweden). Total moisture was determined gravimetrically by loss on drying at 100 °C in an Isotemp® 516 G oven (Fisher Scientific, Waltham, Massachusetts, USA). Fat determination involved continuous extraction by petroleum ether in a Soxhlet apparatus, AOAC Method 960.39 (AOAC, 2000 b). Ash was determined gravimetrically following combustion in a Thermolyne® 6000 muffle furnace (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 550 °C. Carbohydrate was then calculated by difference.

Total dietary fiber was determined gravimetrically following enzymatic digestion with α-amylase (95 °C, 15 min), protease (60 °C, 30 min, pH 7.5), amyloglucosidase (60 °C, 30 min, pH 4.0–4.7), precipitation with 95% ethanol, and filtration through acid washed celite with ethanol and acetone solutions (AOAC, 2000c). Prior to calculation of the final fiber content, the protein content was determined by the Kjeldahl method and subtracted. Enzymes and reagents were purchased from Sigma-Aldrich Corporation (St Louis, Missouri, USA).

Fructose, glucose, and sucrose contents were determined by HPLC according to AOAC method 982.14 (AOAC, 2000d), using standards from Sigma-Aldrich Corp. and by separation with an Agilent 1100 Series LC and refractive index detector (Agilent Technologies, Santa Clara, California, USA). Prior to chromatographic separation, samples were diluted with ethanol:water (1:1, v:v) and heated to 85 °C for 25 min. Samples were then centrifuged and filtered through a 0.45 μm nylon syringe filter and injected into a 5 μm amino column. The mobile phase was acetonitrile:water (80:20, v:v) with a flow rate of 1.5 mL min^{-1}.

Minerals were determined by inductively coupled plasma (ICP) emission spectrometry (AOAC, 2000e, f). Samples were ashed and then treated with concentrated nitric and hydrochloric acids. The treated samples were then analyzed using an Optima 2000 DV optical emission spectrometer (PerkinElmer, Waltham, Massachusetts, USA). AccuTrace™ mineral reference standards (AccuStandard, New Haven, Connecticut, USA) were used to develop calibration curves at the appropriate wavelengths.

Vitamin A, as β-carotene, was determined by a modified AOAC official method 941.15 for an HPLC system (AOAC, 2000g). Briefly, samples were extracted with chloroform, followed by successive partitioning with n-hexane. The organic solvent was removed from the extracted residue by evaporation under nitrogen at 55 °C. The residue was then dissolved in propanol and injected into a Waters 2690 separations module coupled with a 996 Photodiode Array (PDA) detector, equipped with a C8 column (4.6 mm × 250 mm; 5 μm, Waters Corporation, Milford, Massachusetts, USA). The mobile phase was water:propanol (40:60, v:v) with a flow rate of 1 mL min^{-1}.

Vitamin C was determined by titration with 2,6-dichloroindophenol, by the microfluorometric method, or by HPLC and UV detection of oxidized ascorbic acid (AOAC, 2000h, i). Noni fruit puree was filtered, diluted with metaphosphoric acid–acetic acid solution (0.03%:0.08%), and filtered once more. This diluted samples were then titrated with 2,6-dichloroindophenol (1.1 M) and the results calculated based upon the amount consumed, having been previously calibrated with ascorbic acid standard solutions. Alternately, the diluted samples (100 mL) were treated with 2 g acid-washed decolorizing carbon (Norit®, Norit N.V., Amersfoort, Netherlands). 5 mL aliquots of the Norit®-treated samples were diluted 1:1 with a 3% boric acid, in saturated sodium acetate, and the fluorescence read at 430 nm. For the HPLC analysis, the samples were filtered, diluted with 0.01 M sodium heptane sulfonate, and injected into a Waters 2690 separations module coupled with 996 PDA detectors, quipped with a C18 column (4.6 mm × 150 mm; 5 μm). The pump was connected to two mobile phases: A 15 mM phosphate buffer (pH 3.5), and B; methanol. The elution flow rate was 0.75 mL min^{-1}, with a column temperature of 10 °C. The mobile phase was programmed consecutively in linear gradients as follows: 0–5 min, 95% A and 5% B; 5–10 min, 90% A and 10% B; 10–15 min, 85% A and 15% B; 15–30 min, 80% A and 20% B. The PDA detector was monitored in the range of 200–300 nm, and quantified at 240 nm.

Niacin, thiamin, riboflavin, vitamin B6, vitamin B12, folic acid, biotin, and pantothenic acid were determined by AOAC and United States Pharmacopoeia methods (AOAC, 2000j, k, l, m. n, o, p; United States Pharmacopeia, 2005; Scheiner & De Ritter, 1975). Following incubation of samples with the appropriate inoculum and growth media solutions, as per AOAC methods, turbidity was measured with the Autoturb^3 microbiological assay system (Shaefer Technologies, Indianapolis, Indiana, USA) to determine niacin, vitamin B12, biotin,

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and folic acid content. For folic acid determination, the puree samples were hydrolyzed in potassium phosphate buffer, treated with folate conjugase (Difco Laboratories, Detroit, Michigan, USA), followed by incubation with *Lactobacillus casei* (ATCC, Manassas, Virginia, USA) at 37 °C for 22 h. For biotin determination, samples were filtered and incubated with *Lactobacillus plantarum* (ATCC). For niacin determination, samples were also incubated with *L. plantarum*, but were first hydrolyzed with sulfuric acid. Puree samples were diluted with 0.1 M sodium phosphate buffer and autoclaved at 123 °C for 10 min then incubated with *Lactobacillus delbrueckii* (ATCC) to determine vitamin B12 content. For riboflavin, thiamin, pantothenic acid, and vitamin B6 determinations, samples were extracted with 5% acetonitrile, containing 0.6 N acetic acid, and then filtered. The extracts were loaded into an Agilent 1100 Series HPLC system, with a C18 column, and eluted with an isocratic mobile phase of methanol:glacial acetic acid: water (27:1:73, v:v:v) at a flow rate of 1 mL min^{-1}. Analytes were detected and quantified at 270 nm, with the exception of pantothenic acid, which was quantified at 210 nm.

Vitamin E was determined by HPLC similar to a previously reported method (Omale and Omajali, 2010), with modifications. The samples were filtered and extracted directly with n-hexane. The hexane was removed by evaporation and the sample redissolved in propanol. Separation was carried out with a Waters 2690 separations module coupled with a 996 Photodiode Array (PDA) detector and a C8 column (4.6 mm × 250 mm; 5 μm, Waters Corp), with detection at 210 nm. The mobile phase was 2-propanol:H_20 (60:20, %:%), with a flow rate of 1 mL min^{-1}.

Vitamin K was determined according to AOAC method 992.27 (AOAC, 2000p), using an Agilent 1100 Series LC with a UV detector, following extraction of samples with dichloromethane and isooctane (2:1, v:v). The mobile phase was 30% dichloromethane and 0.02% isopropanol in isooctane, with a flow rate of 1 ml min^{-1}. Vitamin K concentration was determined at 254 nm. Amino acids were determined with a Beckman 7300 automated amino acid analyzer (Beckman Coulter, Inc. Fullerton, California), following hydrolysis with 6 M hydrochloric acid for 24 h at 110 °C. For methionine and cystine, the samples (0.1 g) were first treated with 2 mL 88% performic acid overnight at 5 °C. The tryptophan analysis involved hydrolysis with 4.2 M sodium hydroxide for 22 h at 110 °C (AOAC, 2000q). Amino acid standards were obtained from Beckman Coulter, Inc. Biotin, folic acid, vitamin B12, niacin, and vitamin K standards were obtained from the United States Pharmacopeia (Rockville, Maryland). The remaining vitamin standards were obtained from Sigma-Aldrich Corporation.

The iridoid content, inclusive of deacetylasperulosidic acid (DAA) and asperulosidic acid (AA), was determined by HPLC, according to a previously reported method (Deng et al., 2011). Briefly, puree samples were diluted with water:methanol (1:1), mixed thoroughly, and filtered. Filtered samples were collected in 5 mL volumetric flasks for HPLC analysis. Chromatographic separation was performed on a Waters 2690 separations module coupled with a 996 PDA detector, equipped with a C18 column (4.6 mm × 250 mm; 5 μm). The pump was connected to two mobile phases: A; acetonitrile, and B; 0.1% formic acid in water (v:v), and eluted at a flow rate of 0.8 mL min^{-1}. The mobile phase was programmed consecutively in linear gradients as follows: 0–5 min, 0% A; and 40 min, 30% A. The PDA detector was monitored in the range of 210–400 nm. The injection volume was 10 μL for each of the sample solutions. The column temperature was maintained at 25 °C.

Other significant secondary metabolites, such as scopoletin, rutin, and quercetin, were also determined by HPLC (Deng, West, and Jensen, 2010). Noni fruit puree sample preparation was the same as that for iridoid analyses. Chromatographic separation was performed on a Waters 2690 separations module coupled with a 996 PDA detector, and equipped with an Atlantis® C18 column (4.6 mm × 250 mm; 5 μm). The pump was connected to a mobile phase system composed of three solvents: A; acetonitrile, B; methanol, and C; 0.1% trifluoroacetic acid in water. The mobile phase was programmed consecutively in linear gradients as follows: 0 min, 10% A, 10% B, and 80% C; 15 min, 20% A, 20% B, and 60% C; 26 min, 40% A, 40% B, and 20% C; 28–39 min, 50% A, 50% B, and 0% C; and 40–45 min, 10% A, 10% B, and 80% C. The elution was run at a flow rate of 1.0 mL min^{-1}. The UV spectra were monitored at 210 nm, 450 nm, and 365 nm for quantitative analysis. The injection volume was 50 μL for each of the sample solutions. The column temperature was maintained at 25 °C. Reagents for the phytochemical analyses were obtained from Sigma-Aldrich Corporation and Fisher Scientific.

2.3. Acute toxicity test of iridoids

Twenty healthy Sprague–Dawley rats (10 males, 10 females, body weight 181–205 g) were selected for the tests. An iridoid enriched fruit extract was dissolved in water to produce a total iridoid concentration of 8.5 mg/mL. A dose of 340 mg total iridoids/kg body weight (bw) was given to each animal by gastric intubation (20 mL/kg bw twice per day). For 14 days following the administration of the iridoid solution, animals were observed daily for occurrences of death and symptoms of toxicity, including convulsions, irregular breathing, piloerection, and paralysis. As decreased weight is a typical symptom of toxicity, body weights were recorded for each animal on days 0 and 14. The acute toxicity test was carried out in accordance with EC Directive 86/609/EEC (European Communities, 1986).

2.4. Primary DNA damage test in E. coli PQ37

The SOS-chromotest in *E. coli* PQ37 was used to determine the potential for DAA and AA to induce primary DNA damage. This test was carried out according to the previously developed method (Fish et al., 1987). DAA and AA were isolated from noni fruits from Tahiti and purified to >98%. *E. coli* PQ37 was incubated in LB medium for 12 h at 37 °C to reach the exponential growth phase. An aliquot of this culture was diluted with fresh LB medium to $OD_{600} = 0.05$. Aliquots of the diluted *E. coli* PQ37 suspension were incubated in a 96-well plate at 37 °C in the presence of DAA or AA for 2 hours. The DAA and AA concentrations tested were 7.81, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 μg mL^{-1}. Samples were evaluated in triplicate. Following incubation with the samples, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside was added to the wells to detect β-galactosidase enzyme activity, which is induced during SOS repair of damaged DNA. Nitrophenyl phosphate is also added to the wells to measure alkaline phosphatase activity, an indicator of cell viability. The samples were again incubated for 90 min and the absorbances of the samples, blanks and controls were measured at 410 and 620 nm with a microplate reader. Vehicle blanks and positive controls, 1.25 μg mL^{-1} 4-nitroquinoline 1-oxide (4NQO), were included in this test. The induction factor of each material was calculated by dividing the absorbance of the sample at 620 nm by that of the blank, while also correcting for cell viability. Induction factors less than two indicate an absence of genotoxic activity.

2.5. Anti-genotoxicity test in E. coli PQ37

The primary DNA damage test was performed again, similar to the method described above. However, the method was modified to include incubation of *E. coli* PQ37 in the presence of both 1.25 μg mL^{-1} 4NQO and 250 μg mL^{-1} DAA or AA. 250 μg mL^{-1} represented the lower mid-range of concentrations evaluated in the genotoxicity test. Therefore, it was selected for screening of anti-genotoxic activity. Induction factors were calculated in the same manner as described above. The percent reduction in genotoxicity was determined by dividing the difference between the induction factor of 4NQO and the blank (induction factor of 1) by the difference between the induction factor of 4NQO plus DAA or AA and the blank. In this test, as well as in

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the primary DNA damage tests, comparisons were made with Student's t-test.

2.6. Statistical analyses

Means and standard deviations were calculated for each set of analytical results obtained from the different batches. In both the primary DNA damage test and the anti-genotoxicity test, intergroup comparisons were made with Student's t-test.

3. Results and discussion

The nutrient composition of processed noni fruit puree is summarized in Table 1. Proximate nutritional parameters are within the typical ranges for fruits in general. Processed noni fruit puree contains 2 g 100 g^{-1} dietary fiber. Noni fruit does not contain a significant quantity of protein or fat. However, all but one essential amino acid, tryptophan, as well as histidine, essential for infants, were detected in the puree (Table 2). Aspartic acid was the most predominant amino acid. The protein content of the processed puree from French Polynesia is within the same range as that reported for raw noni fruit from Pohnpei, but is much less than that reported for pressed juice from Cambodian noni fruit (Shovic & Whistler, 2001; Chunhieng et al., 2005). Cambodian noni juice is reported to contain 2.5% protein, about five times greater than that reported in processed puree or raw Pohnpei fruit. However, this is an unusually high quantity for a fruit juice. The protein content of purple passion fruit (2.2%) is one of the highest known among fruits, and, by comparison, the juice pressed from passion fruit contains 0.39 g 100 g^{-1} (USDA, 2009). Therefore, it is possible that the high protein content in Cambodian noni juice is reported in error.

The fructose, glucose and sucrose content ranges of processed noni fruit puree are inclusive of the amounts reported for Cambodian noni fruit juice, but the dietary fiber content is slightly less (Chunhieng et al., 2005). The fat content is essentially the same in both the puree and the Cambodian juice, but is reportedly twice as much (0.3 g 100 g^{-1}) in Pohnpei noni fruit (Shovic & Whistler, 2001).

Vitamin C is the most prominent vitamin in noni fruit puree, with a mean content of 1.13 mg^{-1} g. At this concentration, 100 g of puree provides 251% of the recommended daily vitamin C requirement for adults (FAO/WHO, 2001). The reported vitamin C contents of Australian and Pohnpei noni fruits fall within the range observed in the noni fruit puree (Shovic & Whistler, 2001; Chunhieng et al., 2005). Vitamin A in raw Pohnpei noni fruit is reported as undetectable, <3.5 retinol equivalents (RE) 100 g^{-1} (Shovic & Whistler, 2001). However, noni fruit puree from French Polynesia is found to contain appreciable quantities of β-carotene. As calculated from β-carotene concentration, the mean vitamin A content per 100 g of puree is 318.17 RE. The joint FAO/WHO recommendation for average vitamin A daily intake by adults is 270 RE for females and 300 RE for males (FAO/WHO, 1988). As such, noni fruit puree appears to have the potential to be a significant dietary source of vitamin A. The niacin content of processed noni fruit is great enough to have some nutritional impact, but will only be significant when larger quantities are consumed. At 100 g, the puree provides 18 to 21% of the recommended niacin intake for adults (FAO/WHO, 2001). Thiamin, riboflavin, vitamin B6, vitamin B12, folic acid, pantothenic acid, and vitamin K were below detection limits. Processed noni fruit puree contains, but is not a significant source of, vitamin E and biotin.

Potassium appears to be the most abundant mineral in processed noni fruit puree. This is also consistent with mineral analyses performed for Cambodian noni fruit juice and raw noni fruit from Pohnpei and Australia (Shovic & Whistler, 2001; Chunhieng et al., 2005; Peerzada et al., 1990). In the processed puree, it is more than four times the concentration of calcium, the next most abundant mineral, although neither is present in nutritionally significant quantities. The mean calcium content of the puree is greater than the amounts reported for the other sources of noni fruit, with the exception of a minimal difference between Pohnpei fruit (48.2 mg 100 g^{-1} vs. 41.7 mg 100 g^{-1}). Only two minerals are present in nutritionally significant amounts. In 100 g of noni puree, manganese and selenium contents would meet approximately 18 to 26% of the recommended daily allowance for adults (Institute of Medicine, 2000, 2001). The average manganese content of the processed puree, 0.47 mg 100 g^{-1}, is five times greater than the amount reported in raw Pohnpei noni fruit, 0.094 mg 100 g^{-1} (Shovic & Whistler, 2001). Selenium concentration has only been reported previously for Cambodian noni fruit juice, with an amount equivalent to what is observed in the processed puree (Chunhieng et al., 2005).

Table 1
Nutrient content of processed noni fruit puree.

Assay	Mean	S.D.
Protein (g/100 g)	0.55	0.11
Fat (g/100 g)	0.10	0.12
Moisture (g/100 g)	91.63	1.98
Ash (g/100 g)	0.54	0.19
Carbohydrate (g/100 g)	7.21	1.81
Fructose (g/100 g)	1.07	0.39
Glucose (g/100 g)	1.30	0.36
Sucrose (g/100 g)	<0.1	-
Kilojoules/100 g	135.56	31.73
Dietary fiber (g/100 g)	2.01	0.27
Ca (mg/100 g)	48.20	16.04
K (mg/100 g)	214.34	56.91
Na (mg/100 g)	16.99	5.98
Mg (mg/100 g)	26.10	8.33
P (mg/100 g)	20.35	6.78
Fe (mg/100 g)	0.74	0.06
Cu (mg/100 g)	0.08	0.07
Mn (mg/100 g)	0.47	0.62
Se (mg/100 g)	0.01	0.01
Zn (mg/100 g)	0.06	0.07
β-carotene (μg/g)	19.09	12.15
Niacin (mg/g)	0.03	0.01
Vitamin C (mg/g)	1.13	0.77
Thiamin (mg/g)	<0.018	–
Riboflavin (mg/g)	<0.018	–
Vitamin B6 (mg/g)	<0.018	–
Vitamin B12 (μg/g)	<0.0012	–
Vitamin E (μg/g)	10.96	6.62
Folic acid (μg/g)	<0.06	–
Biotin (μg/g)	0.02	0.00
Pantothenic acid (mg/g)	<0.018	–
Vitamin K (μg/g)	<0.10	–

S.D. = standard deviation.

Table 2
Amino acid profile of processed noni fruit puree.

Amino acid	Mean	S.D.
Alanine (mg/g)	0.45	0.04
Arginine (mg/g)	0.32	0.04
Aspartic acid (mg/g)	0.80	0.08
Cystine (mg/g)	0.23	0.03
Glutamic acid (mg/g)	0.64	0.05
Glycine (mg/g)	0.36	0.04
Histidine (mg/g)	<0.1	–
Isoleucine (mg/g)	0.29	0.01
Leucine (mg/g)	0.38	0.02
Lysine (mg/g)	0.25	0.04
Methionine (mg/g)	<0.1	–
Phenylalanine (mg/g)	0.21	0.05
Proline (mg/g)	0.26	0.03
Serine (mg/g)	0.27	0.02
Threonine (mg/g)	0.27	0.03
Tryptophan (mg/g)	<0.1	0.00
Tyrosine (mg/g)	0.25	0.03
Valine (mg/g)	0.36	0.03

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The differences in mineral contents between the different sources of noni could be due to stage of ripeness at harvest, climate, soil conditions, and genetic variability between the sources (Cunningham et al., 2001; Razafimandimbison et al., 2010).

The phytochemical analyses reveal that iridoids are the major secondary metabolites produced by noni fruit and are present in significant quantities following processing (Table 3). The DAA content of the puree is similar to that of juice pressed from raw noni fruits from French Polynesia, but the mean AA content is 1.8 times greater than that of the pressed juice, 38.79 mg 100 g^{-1} vs. 21.80 mg 100 g^{-1} (Deng et al., 2011). The average DAA content, dry weight basis, of the processed puree is approximately 6.5 times higher than that reported in commercial ground noni fruit powder from Hawaii, and 2.3 times greater than ripe noni fruit grown in a botanical garden in Switzerland (Potterat et al., 2007). Comparing against the same samples, the mean AA concentration in the puree is 2.9 and 5.5 times greater than in ground noni fruit powder and the botanical garden fruit, respectively. The differences observed between the botanical garden sample and the puree are likely due to differences in growing environments. The lower iridoid levels in the commercial fruit powder from Hawaii might be due to aggressive drying methods at elevated temperatures, but additional research would be required to confirm this.

Scopoletin, rutin, and quercetin were also present after processing (Table 3). The scopoletin content of the puree falls within the range reported for juice pressed from raw noni fruits from 14 different islands in the Pacific and Indian Oceans (Basar & Westendorf, 2010). The rutin content of the puree falls within the lower range reported for some commercial noni juice samples, but the quercetin content is almost double that of the highest amount reported in any of these samples (Deng, West, & Jensen, 2010). Quercetin is an aglycone of rutin, and its increased concentration in the puree is likely the result of rutin glycolysis during processing and pasteurization (Rohn et al., 2007).

In the puree, the total iridoid content was 20 times greater than the combined concentrations of the other three phytochemicals. Deacetylasperulosidic acid accounted for 78% of the total iridoid content. Due to their prevalence in noni fruit, both iridoids may be used as markers for identification of products containing authentic noni ingredients. The reported bioactivities of iridoids correlate well to several of the *in vitro* and *in vivo* bioactivities reported for noni fruit juice and noni fruit extracts, including antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective, and hypolipidemic activities (Tundis et al., 2008; Wang et al., 2002). As the major phytochemical in processed noni fruit, deacetylasperulosidic acid should be investigated further for its role in the potential health benefits observed in human clinical trials of noni fruit juice (Wang, Peng, et al., 2009, Wang, Lutfiyya, et al., 2009; Palu et al., 2008; Akinbo et al., 2006; Langford et al., 2004; Ma et al., 2008).

No deaths or symptoms of toxicity were observed in the acute toxicity test. Animals also gained appropriate weight (Table 4). The LD_{50} of noni iridoids was determined to be >340 mg/kg bw. In the primary DNA damage test in *E. coli* PQ37 (Table 5), the mean induction factors for DAA and AA, at 1000 μg mL^{-1}, were 1.07 and 1.09, respectively. At all concentrations tested, DAA and AA did not induce any SOS repair at a frequency significantly above that of the blank. Statistically, induction factors were no different than that of the blank, and all results remained well below the two-fold criteria for genotoxicity. SOS-chromotest results have a high level of agreement (86%) with those from the reverse mutation assay (Legault et al. 1994). Therefore, the SOS-chromotest has some utility in predicting potential mutagenicity, in addition to primary DNA damage. The lack of DAA and AA toxicity in these tests is consistent with the results of toxicity tests of noni fruit juice (West, White, et al., 2009; West, Su and Jensen, 2009; Westendorf et al., 2007).

In the anti-genotoxicity test, 4NQO, exhibited obvious genotoxicity, inducing SOS repair more than 8-fold above that of the vehicle blank. But the induction factors of 4NQO plus DAA or AA, were the same as those of DAA or AA alone (Table 6), with no statistical difference from that of the vehicle blank. The reductions in genotoxicity from 250 μg mL^{-1} DAA and AA were 98.96 and 99.22%, respectively. Therefore, the genotoxic activity of 4NQO was almost entirely abolished by the addition of either iridoid.

A double-blind human clinical trial revealed that ingestion of noni fruit juice reduced the amount of aromatic DNA-adduct formation in the lymphocytes of current heavy cigarette smokers (Wang, Lutfiyya, et al., 2009). 4NQO exhibits genotoxic activity in *E. coli* through the formation of 4NQO-guanine and 4NQO-adenine adducts (Ikenaga et al., 1975; Thomas et al., 1991). These DNA lesions lead to the induction of the SOS repair mechanism. As such, the reduction in 4NQO genotoxicity by DAA and AA equates to a reduction in DNA adduct formation. Therefore, the results of the current anti-genotoxicity test suggest the possible involvement of these iridoids in noni juice's DNA protective effects.

Table 3
Phytochemical content of processed noni fruit puree.

Assay	Mean	S.D.
Deacetylasperulosidic acid (mg/100 g)	137.61	13.69
Asperulosidic acid (mg/100 g)	38.79	9.18
Scopoletin (mg/100 g)	5.68	1.58
Rutin (mg/100 g)	1.42	0.84
Quercetin (mg/100 g)	1.59	0.71

Table 4
Acute toxicity test of noni iridoids.

Animal	Sex	Animal number	Body weight (g)		LD_{50} (mg iridoids/kg bw)
			Before	After	
S.D. rat	Male	10	191.2 ± 5.9	216.1 ± 8.3	>340.0
	Female	10	192.8 ± 12.3	289.4 ± 12.3	>340.0

4. Conclusion

Processed noni fruit puree is a potential dietary source of vitamin C, vitamin A, niacin, manganese, and selenium. Vitamin C is the major nutrient present, in terms of concentration. The major phytochemicals in the puree are iridoids, especially DAA. The iridoids in noni did not display any toxicity. On the other hand, these iridoids did display potential anti-genotoxic activity. Even though processed noni fruit puree contained an appreciable quantity of vitamin C, the average DAA content was approximately 22% greater than that of vitamin C.

Table 5
Primary DNA damage assay in *E. coli* PQ37.

Compound	Concentration (μg mL^{-1})	Induction factor
Deacetylasperulosidic acid	1000	1.07 ± 0.14
	500	1.03 ± 0.02
	250	1.06 ± 0.06
	125	1.00 ± 0.08
	62.5	1.05 ± 0.07
	31.2	1.04 ± 0.08
	15.6	1.03 ± 0.16
	7.81	0.93 ± 0.13
Asperulosidic acid	1000	1.09 ± 0.03
	500	1.07 ± 0.04
	250	1.11 ± 0.16
	125	1.02 ± 0.08
	62.5	1.04 ± 0.13
	31.2	0.99 ± 0.06
	15.6	1.04 ± 0.11
	7.81	1.01 ± 0.05
4NQO	1.25	8.69 ± 3.69*

*P<0.05, compared to vehicle blank.

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Table 6
Anti-genotoxicity test in *E. coli* PQ37.

Compound	Concentration (μg mL^{-1})	Induction factor
Positive control (4NQO)	1.25	8.69 ± 3.69**
4NQO + deacetylasperulosidic acid	250*	1.08 ± 0.12
4NQO + asperulosidic acid	250*	1.06 ± 0.03

*DAA or AA concentration; 4NQO concentration is 1.25 μg mL^{-1}.
**P<0.05, compared to vehicle blank.

These findings suggest that DAA may play an important role in the biological activities of noni fruit juice that have been observed *in vitro*, *in vivo*, and in human clinical trials.

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References

Akinbo, S. R. A., Noronha, C. C., Okanlawonand, A. O., & Danesi, M. A. (2006). Comparative study of the effect of Morinda citrifolia (noni) with selected physiotherapy modalities in the management of patients with cervical spondylosis. *Nigerian Journal of Health and Biomedical Sciences*, *5*, 6−11.

AOAC (Association of Official Analytical Chemists) (2000a). *Official method, 979.09, Protein (crude) in animal feed. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000b). *Official method, 960.39, Fat (crude) in meat. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000c). *Official method, 991.43, Total, soluble, and insoluble dietary fiber. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000d). *Official method, 982.14, Glucose, fructose, sucrose, and maltose in presweetened cereals. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000e). *Official method, 985.01, Metals and other elements in plants and pet foods. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000f). *Official method, 948.27, Sediment in dairy products. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000g). *Official method, 941.15, Carotene in fresh plant material and silages. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000h). *Official method, 967.21, Ascorbic acid in vitamin preparation and juices, 2, 6-dichloroindophenol titrimetric method. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000i). *Official method, 967.22, Vitamin C (total) in vitamin preparations, microfluorometric method. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000j). *Official method, 944.13, Niacin and niacinamide (nicotinic acid and nicotinamide) in vitamin preparations. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000k). *Official method, 985.34, Niacin and niacinamide (nicotinic acid and nicotinamide) in ready-to-feed milk-based infant formula. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000l). *Official method, 952.20, Cobalamin (vitamin B12 activity) in vitamin preparations, microbiological methods. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000m). *Official method, 960.46, Vitamin assays, microbiological methods. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000n). *Official method, 960.45, Vitamin A in margarine, spectrophotometric method. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000o). *Official method, 992.05, Folic acid (pteroylglutamic acid) in infant formula, microbiological methods. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000p). *Official method, 992.27, Trans-vitamin K1 (phylloquinone) in ready-to-feed milk-based infant formula, liquid chromatographic method. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

AOAC (Association of Official Analytical Chemists) (2000q). *Official method, 982.30, Protein efficiency ratio. In, Official methods of analysis of the association of official analytical chemists* (17th ed). Gaithersburg, MD: AOAC International.

Basar, S., & Westendorf, J. (2010). Identification of (2E,4Z,7Z)-decatrienoic acid in noni fruit and its use in quality screening of commercial noni products.*Food Analytical Methods*, doi:10.1007/s12161-010-9125-9 published online 23 February 2010.

Chunhieng, T., Hay, L., & Montet, D. (2005). Detailed study of the juice composition of noni (*Morinda citrifolia*) fruits from Cambodia. *Fruits*, *60*, 13−24.

Communities, European (1986). Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.*Official Journal L*, *358*, 1−28 18.12.1986.

Communities, European (1997). Regulation 258/97/EC of the European Parliament and of the Council of the 27 January 1997 concerning novel foods and novel food ingredients. *Official Journal of the European Communities L*, *43*(40), 1−6.

Cunningham, J. H., Milligan, G., & Trevisan, L. (2001). *Minerals in Australian fruits and vegetables — a comparison of levels between the 1980s and 2000*. Canberra: Food Standards Australia New Zealand.

Deng, S., West, B. J., & Jensen, J. (2010). A quantitative comparison of phytochemical components in global noni fruits and their commercial products. *Food Chemistry*, *122*, 267−270.

Deng, S., West, B. J., Palu, A. K., & Jensen, C. J. (2011). Determination and comparative analysis of major iridoids in different parts and cultivation sources of *Morinda citrifolia*. *Phytochemical Analysis*, *22*(1), 26−30. Published online 26 August 2010.

DGSANCO (Health and Consumer Protection Directorate General) (2010). List of notifications of novel foods.http://ec.europa.eu/food/food/biotechnology/novelfood/notif_list_en.pdf Accessed 17 June 2010. Brussels: European Commission.

European Commission (2003). Commission decision of 5 June 2003 authorising the placing on the market of "noni juice" (juice of the fruit of *Morinda citrifolia* L.) as a novel food ingredient under regulation (EC) No 258/97 of the European parliament and of the council. *Official Journal of the European Union L*, *144*(46), 12.

European Commission (2010). Commission Decision of 21 April 2010 authorising the placing on the market of puree and concentrate of the fruits of *Morinda citrifolia* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. *Official Journal of the European Union L*, *102*(51), 49−51.

FAO/WHO (1988). Requirements of vitamin A, iron, folate and vitamin B12. Report of a Joint FAO/WHO Expert Consultation. Rome: Food and Agriculture Organization of the United Nations.

FAO/WHO (2001). Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation in Bangkok, Thailand. Rome: Food and Agricultural Organization of the United Nations.

Fish, F., Lampert, I., Halachmi, A., Riesenfeld, G., & Herzberg, M. (1987). The SOS chromotest kit: A rapid method for the detection of genotoxicity. *Toxicity Assessment*, *2*, 135−147.

Ikenaga, M., Ichikawa-Ryo, H., & Kondo, S. (1975). The major cause of inactivation and mutation by 4-nitroquinoline 1-oxide in *Escherichia coli*: Excisable 4NQO-purine adducts. *Journal of Molecular Biology*, *92*, 341−356.

Institute of Medicine, Food and Nutrition Board (2000). *Dietary reference intakes: Vitamin C, vitamin E, selenium, and carotenoids*. Washington, D.C.: National Academy Press.

Institute of Medicine, Food and Nutrition Board (2001). *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington, D.C.: National Academy Press.

Kamiya, K., Tanaka, Y., Endang, H., Uma, M., & Satake, T. (2005). New anthraquinone and iridoid from the fruits of *Morinda citrifolia*. *Chemical & Pharmaceutical Bulletin*, *53*, 1597−1599.

Langford, J., Doughty, A., Wang, M., Clayton, L., & Babich, M. (2004). Effects of *Morinda citrifolia* on quality of life and auditory function in postmenopausal women. *Journal of Alternative and Complementary Medicine*, *10*, 737−742.

Legault, R., Blaise, C., Rokosh, D., & Chong-Kit, R. (1994). Comparative assessment of the SOS chromotest kit and the mutatox test with the *Salmonella* plate incorporation (Ames test) and fluctuation tests for screening genotoxic agents. *Environmental Toxicology and Water Quality*, *9*, 45−57.

Ma, D., Jun, Z., Jianhua, G., et al. (2008). The effect of Tahitian noni juice on antioxidation and immune function. *Chinese Medical Research & Clinical*, *6*, 8−10.

Morton, J. F. (1992). The ocean going noni, or Indian Mulberry (*Morinda citrifolia*, Rubiaceae) and some of its 'colorful' relatives. *Economic Botany*, *46*, 241−256.

Murcia, M. A., Jimenez, A. M., & Martinez-Tome, M. (2009). Vegetables antioxidant losses during industrial processing and refrigerated storage. *Food Research International*, *42*, 1046−1052.

Omale, J., & Omajali, J. B. (2010). Studies on some nutritional characteristics of the fruit and leaf of *Saba florida* (Benth.) from Ibaji forest.. *International Journal of Nutrition and Metabolism*, *2*, 11−26.

Palu, A. K., Seifulla, R. D., & West, B. J. (2008). *Morinda citrifolia* L. (noni) improves athlete endurance: Its mechanisms of action. *Journal of Medicinal Plant Research*, *2*, 154−158.

Peerzada, N., Renaud, S., & Ryan, P. (1990). Vitamin C and elemental composition of some bushfruits. *Journal of Plant Nutrition*, *13*, 787−793.

Author's personal copy

Potterat, O., Felten, R. V., Dalsgaard, P. W., & Hamburger, M. (2007). Identification of TLC markers and quantification by HPLC-MS of various constituents in noni fruit powder and commercial noni-derived products. *Journal of Agricultural and Food Chemistry*, *55*, 7489−7494.

Razafimandimbison, S. G., McDowell, T. D., Halford, D. A., & Bremer, B. (2010). Origin of the pantropical and nutriceutical *Morinda citrifolia* L. (Rubiaceae): Comments on its distribution range and circumscription. *Journal of Biogeography*, *37*, 520−529.

Rodrigues, A. S., Perez-Gregorio, M. R., Garcia-Falcon, M. S., & Simal-Gandara, J. (2009). Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs. *Food Research International*, *42*, 1331−1336.

Rohn, S., Buchner, N., Driemel, G., Rauser, M., & Kroh, L. W. (2007). Thermal degradation of onion quercetin glucosides under roasting conditions. *Journal of Agricultural and Food Chemistry*, *55*, 1568−1573.

Scheiner, J., & De Ritter, E. (1975). Biotin content of feedstuffs. *Journal of Agricultural and Food Chemistry*, *23*, 115−1162.

Shovic, A. C., & Whistler, W. A. (2001). Food sources of provitamin A and vitamin C in the American Pacific. *Tropical Science*, *41*, 199−202.

Thomas, D. C., Husain, I., Chaney, S. G., Panigrahi, G. B., & Walker, I. G. (1991). Sequence effect on incision by (A)BC excinuclease of 4NQO adducts and UV photoproducts. *Nucleic Acids Research*, *19*, 365−370.

Tundis, R., Loizzo, M. R., Menichini, F., Statti, G. A., & Menichini, F. (2008). Biological and pharmacological activities of iridoids: Recent developments. *Mini Reviews in Medicinal Chemistry*, *8*, 399−420.

United States Pharmacopeia (2005). *The United States pharmacopeia, 28th rev., and the national formulary* (23rd ed). Rockville, MD: United States Pharmacopeial Convention.

USDA (United States Department of Agriculture) (2009). Passion-fruit juice, purple, raw. USDA National Nutrient Database for Standard Reference, Release 22, Online.http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl Accessed 16 September 2010.

Wang, M. Y., Lutfiyya, M. N., Weidenbacher-Hoper, V., Anderson, G., Su, C. X., & West, B. J. (2009). Antioxidant activity of noni juice in heavy smokers. *Chemistry Central Journal*, *3*(13), 1−5.

Wang, M. Y., Peng, L., Lutfiyya, M. N., Henley, E., Weidenbacher-Hoper, V., & Anderson, G. (2009). *Morinda citrifolia* (noni) reduces cancer risk in current smokers by decreasing aromatic DNA adducts. *Nutrition and Cancer*, *61*, 634−639.

Wang, M. Y., & Su, C. (2001). Cancer preventive effect of *Morinda citrifolia* (Noni). *Annals of the New York Academy of Sciences*, *952*, 161−168.

Wang, M. Y., West, B. J., Jensen, C. J., Nowicki, D., Su, C., Palu, A. K., et al. (2002). *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. *Acta Pharmacologica Sinica*, *23*, 1127−1141.

West, B. J., Su, C. X., & Jensen, C. J. (2009). Hepatotoxicity and subchronic toxicity tests of *Morinda citrifolia* (noni) fruit. *The Journal of Toxicological Sciences*, *34*, 581−585.

West, B. J., White, L. D., Jensen, C. J., Palu, A. K., & Westendorf, J. (2009). A double-blind clinical safety study of noni fruit juice. *Pacific Health Dialog*, *15*, 21−32.

Westendorf, J., Effenberger, K., Iznaguen, H., & Basar, S. (2007). Toxicological and analytical investigations of noni (*Morinda citrifolia*) fruit juice. *Journal of Agricultural and Food Chemistry*, *55*, 529−537.