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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.

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 phytochemical 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,