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Identification of (2*E*,4*Z*,7*Z*)-Decatrienoic Acid in Noni Fruit and Its Use in Quality Screening of Commercial Noni Products

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Abstract The fruit of noni (*Morinda citrifolia* L.) has been used traditionally as food and for medicinal purposes for over 2,000 years. Today, hundreds of different brands of noni fruit juices appear on the market. In this study, we provide an analytical method for the quality assurance of noni fruit juices based on the determination of two organic (scopoletin and (2*E*,4*Z*,7*Z*)-decatrienoic acid (DTA)) and one inorganic marker substances (potassium). DTA was observed in noni fruit for the first time. A total of 32 authentic and 16 commercially produced noni fruit juices were investigated. In non-processed authentic noni fruit juices, DTA was almost completely present as a glycoside, whereas considerable amounts of free DTA occurred in commercial juices. Concentration of noni juice by heat evaporation reduces the amount of DTA compared to other non-volatile marker compounds, such as scopoletin. It is therefore possible to distinguish between noni juices prepared with or without fermentation or by rehydration of noni fruit juice concentrates. The degree of dilution of commercial noni fruit juices was also easily detectable by determination of the potassium content, which should be in the range of 1,000–3,000 mg/L, as determined in authentic noni fruit juices.

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Introduction

Noni (*Morinda citrifolia* L.), belonging to the Rubiacea family, is a small woodland tree growing in almost all tropical areas of the world. The robust plant grows as a shrub or tree depending on conditions of soil and climate. On the Polynesian islands and Hawaii, noni plants grow wild as well as in plantations, on beach areas, and inland up to an elevation of 600 m. Flowers and fruit at all phases of ripeness can be observed on the plant year around. Noni plants proliferate very rapidly and bear fruit in the first year after sprouting already.

Noni plants are very important in Polynesian culture. Noni fruit has been used for more than two thousand years as food or as food ingredients (Cheeseman 1903). Even the US Army prepared a list of edible plants on Pacific islands during the Second World War, which also included noni fruit (Merrill 1943). Moreover, in Polynesian folk medicine, noni fruits and leaves have also been used to treat arthritis and infections, heal wounds, stop or reduce pain, and cure colds and several other diseases (Dixon et al. 1999; McClatchey 2002; Wang et al. 2002). During the past decade, juices prepared from ripe noni fruit became very popular worldwide as a wellness drink. In 2003, noni fruit juice was approved as "novel food" by the European Commission (European 2003). No substantial adverse effects of noni fruit juice have been observed in extensive toxicological studies, including tissue culture work, animal experiments, and a human clinical trial (West et al. 2006; Westendorf et al. 2007).

Pure noni fruit juice has a light-brownish color; a characteristic odor, reminiscent of old cheese; and a strong unpleasant taste. Therefore, most products on the market are supplemented with other fruit juices and natural or synthetic flavorings. In many cases, there is no indication on the label regarding the content of pure noni juice in these mixtures.

Today, global yearly consumption of noni fruit juice is more than 60 million liters. Numerous different noni juice products are available on the market, many of which are of poor quality. It is difficult to distinguish between noni juices of high and low quality because official quality criteria for such products are absent. The need for quality control of noni products was first discussed at a Hawaiian Noni Conference in 2002 (Ram 2003). A quantitative HPLC-MS assay for the quality control of noni fruit products has recently been published (Potterat et al. 2007). Although powerful and effective, this method needs special equipment and reference standards.

In the present work, we provide a simple analytical method for the characterization of noni fruit juice products based on the determination of two organic (scopoletin and (2*E*,4*Z*,7*Z*)-decatrienoic acid (DTA)) marker compounds and one inorganic (potassium) constituent. DTA is extremely rare in fruits and, therefore, an ideal choice for the characterization of noni fruit, whereas the concentration of potassium indicates a possible dilution of the juice. The method is easy to perform and highly indicative of the authenticity and quality of noni fruit juices.

Materials and Methods

Chemicals and Reference Compounds

HPLC-grade solvents, *n*-hexane, ethyl acetate (EtOAc), methanol (MeOH), ethanol (EtOH), and water were obtained from Merck (Darmstadt, Germany), as well as trifluoroacetic acid (TFA) for spectroscopy and sulfuric acid (H_2SO_4) (95–97%) and nitric acid (HNO_3) (65%) for analysis. HPLC-grade acetonitrile (MeCN) was purchased from J.T. Baker (Deventer, The Netherlands). The standard compound scopoletin was purchased from ICN Biochemicals Inc. (Heidelberg, Germany) and benzene-d_6 for NMR spectroscopy was supplied from Roth GmbH (Karlsruhe, Germany). An atomic absorption spectroscopy (AAS) standard solution of potassium nitrate (Certipur, 1,000 mg K/L) was purchased from Merck.

Plant Materials

Fresh noni fruit was harvested on the islands of Mauritius and the Maldives (Indian Ocean); Hawaii (Hawaii); Tahiti Moorea, Huahine, Raiatea, and Tahaa (Society Islands, French Polynesia); and Nuku Hiva, Ua Pou, Ua Huka, Hiva Oa, Tahuata, and Fatu Hiva (Marquesas, French Polynesia). The fruits were frozen on site and carried to Hamburg. Freeze-dried noni fruit puree was obtained from Tahitian Noni International (Provo, UT, USA) and used for the isolation of DTA and the production of a reconstituted noni fruit juice.

Thirteen different tropical fruits, pineapple (*Ananas conesus*), avocado (*Persea americana* MILL.), banana (*Musa paradisicia* L.), mirabelle plum (*Prunus domestica*), pomegranate (*Punica granatum* L.), grapefruit (*Citrus paradise*), greengage (*P. domestica* subsp. *Italica*), tuna (*Opuntia ficus-indica*), kiwifruit (*Actinidia deliciosa* Ferg.), mango (*Mangifera indica*), papaya (*Carica papaya*), cape gooseberry (*Physalis peruviana*), and Japanese persimmon (kaki) (*Diospyros kaki*), were obtained from a German supermarket.

Commercial Noni Juices

The following 16 juices have been analyzed: Serrania Bio-Noni fruit juice (Maneva GmbH Naturprodukte, Memmingen, Germany), Noni 99,6% Direktsaft mit Himbeeraroma (Mega Vital Shop, Gablingen, Germany), goodnoni™Noni Saft (Herbex, Lautoka, Fiji), Tahitian Noni Juice (Tahitian Noni International U.K., London, UK), Noni-Saft 100% reiner Direkt-Noni-Saft (Noni Express B.V., Kerkrade, Netherlands), Noni Morinda citrifolia Direktsaft (Hoka GmbH, Königsbach-Stein, Germany), Noni Direktsaft 100% (Vitalität GmbH, Berlin, Germany), Sanct Bernhard Bio-Noni Vitalsaft (Kräuterhaus Sanct Bernhard, Bad Ditzenbach, Germany), Noni Fruchtsaft (Bio) (Govinda Natur GmbH, Abentheuer, Germany), Noni Succo (Dynamic Health, Brooklyn, New York, NY, USA), Noni 100% reiner Direktsaft (Bio) (Sonnenmacht International, Tübingen, Germany), morindafit® Noni Bio Saft (Rainbow Gesellschaft für Naturprodukte mbH, Hamburg, Germany), Noni Morinda citrifolia (Bio) (Kalverton, Tartu, Lithuania), Noni's Best (proV® Nutraceuticals B.V., Vinkeveen, Netherlands), Tahitian Noni N-Core (Tahitian Noni International), Vitalis Noni Saft mit Meeresmineralien (Vitalia Vertriebs GmbH, Würzburg, Germany). Juices were randomly referred to as S1–S16.

Isolation of (2*E*,4*Z*,7*Z*)-Decatrienoic Acid (DTA)

For the isolation of DTA, 100 g of freeze dried and grounded puree of *M. citrifolia* fruits from French Polynesia (Tahitian Noni International) were extracted two times with 200 mL *n*-hexane for 9 h in a rotating incubator at 37°C. After filtration, the solvent was combined and evaporated to dryness under reduced pressure. The dry

extract was dissolved in 5 mL of MeOH. Formation of a white precipitate was observed, which was removed by filtration. The MeOH phase was then concentrated to 1 mL and fractionated on a preparative HPLC system (Merck Hitachi, LaChrom) equipped with a RP-18 column (Hibar RT 250-25, LiChrosorb® RP-18, 7 μm, Merck). Elution was performed with a MeOH–H_2O gradient (0–35 min, 75% MeOH; 35–40 min, 75–100% MeOH; 40–85 min, 100% MeOH; and 85–90 min, 100–75% MeOH) at a flow rate of 5 mL/min. The extract was monitored at 254 nm, where 11 fractions were collected, evaporated to dryness, and then dissolved in 1 mL MeOH subsequently. All fractions together with the original extract were analyzed on an analytical HPLC system (Thermo-Finnigan, Bremen, Germany), equipped with a Nucleosil-100 RP-18 10 μm HPLC column (250×4.6 mm) (Macherey-Nagel, Düren, Germany). A gradient of MeCN–H_2O containing 0.1% TFA at 2 mL/min was used (0–5 min, 20% MeCN; 5–15 min, 20–45% MeCN; 15–20 min, 45% MeCN; 20–30 min, 45–100% MeCN; 30–40 min, 100% MeCN). Fraction 7 was found to contain DTA. Further purification of the compound was performed with the same HPLC system and conditions.

Identification of (2*E*,4*Z*,7*Z*)-Decatrienoic Acid (DTA)

Structure elucidation of DTA was carried out on the basis of MS, 1-D, and 2-D NMR techniques.

The mass spectrum of DTA was determined using a Varian MAT 311 Mass Spectrometer by direct inlet method and by electron impact (70 eV) GC-MS, connected with a Hewlett-Packard HP 5890 gas chromatograph (25 m fused silica capillary with polydimethylsiloxane CPSil-5) coupled to a VG analytical 70–250 S mass spectrometer (ion source temp. 250°C).

The pure DTA, which was isolated as a pale yellow oil, produced a molecular ion signal at *m/z*=166 in its mass spectrum corresponding to $C_{10}H_{14}O_2$. The EI-MS *m/z* (%) of DTA was: 166 (3) [M^+], 137 (3) [M^+–C_2H_5], 123 (10) [M^+–C_3H_7], 121 (23) [M^+–COOH], 97 (32), 91 (53), 81 (31), 79 (100) [$C_6H_7^+$], 77 (46) (Fig. 1). No derivatization of the compound was needed prior to injection.

NMR measurements were carried out with a Bruker WM 400 (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) or a Bruker WM 500 (^{1}H, 500 MHz; ^{13}C, 125.8 MHz) instrument in C_6D_6.

The ^{1}H NMR and ^{13}C NMR absorptions are shown in Table 1. The spectroscopic data were found to be identical to those published earlier (Armin et al. 1999).

Preparation of Pure Noni Fruit Juice from Noni Fruits

Frozen noni fruits were cut into halves and thawed; the juice was squeezed out with a potato press. For direct injections, 500 μL of these juices was centrifuged in Eppendorf cups (13,000 rpm, 3 min) to obtain a clear supernatant for the analysis. Otherwise, all juices, either self-prepared or commercial, were extracted with the pulp material they contain. Calculations of the dry matter content of the juices (in milligrams per milliliter) were performed by freeze drying 100 μL of the juices and weighing of the remaining solid phase.

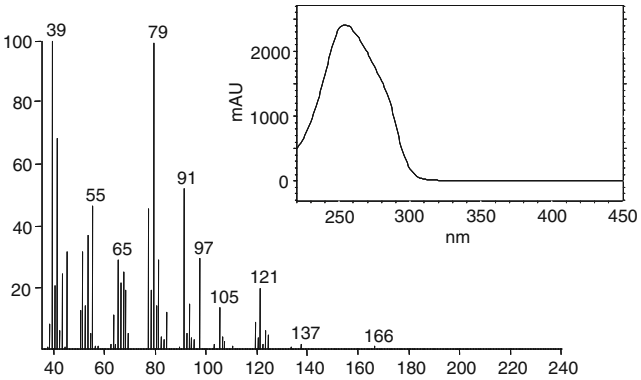

Fig. 1 Mass and UV spectra (at 254 nm) of DTA

HPLC Method for the Quality Assurance of Noni Juices

From each noni juice (self prepared or commercial), 0.5 mL was extracted with 0.5 mL EtOAc by vigorous shaking on a Vortex mixer for 30 s in an Eppendorf cup. After centrifugation (13,000 rpm, 3 min), 250 μL of the organic phase was transferred to another Eppendorf cup and the solvent was evaporated to dryness. The residue was dissolved in 100 μL EtOH; 20 μL was injected on the HPLC column (Nucleosil 100 RP-18 10 μm) and the elution was performed as described before. The marker peaks of scopoletin and DTA were identified by their retention times and UV-spectra. Five independent extractions were performed for every sample. The concentrations of the marker compounds in the juices were calculated in micrograms per milliliter.

Table 1 ^{1}H-^{13}C COSY spectral data for DTA in C_6D_6

Carbon No.	^{13}C (ppm)	^{1}H (ppm)
1	171.50	
2	120.86	5.91 (1H, d, *J*=15.13 Hz)
3	141.5	7.97 (1H, dd, *J*=15.13,15.14 Hz)
4	126.5	5.87 (1H, dd, *J*=11.03 Hz)
5	140	5.60 (1H, dt, *J*=10.41, 7.56 Hz)
6	26.43	2.80 (2H, dd, *J*=7.57, 7.56 Hz)
7	125.5	5.25 (1H, dtt, *J*=10.7, 7.2, 1.6 Hz)
8	133	5.44 (1H, dtt, *J*=10.41, 7.2, 1.6 Hz)
9	20.73	1.96 (2H, m)
10	14.19	0.95 (3H, t, *J*=7.57 Hz)

Hydrolysis of Noni Juices

Hydrolysis was performed on noni juices by mixing 0.5 mL juice and 0.5 mL H_2SO_4 (8%) in an Eppendorf cup, which was heated to 95°C for 20 min in an Eppendorf Heating Block. After 20 min, a dark green color appeared in the mixture. It was left to cool down to room temperature. Next, 500 µL EtOAc was added to the mixture, followed by vigorous shaking on a Vortex mixer for 30 s and then centrifuged (13,000 rpm, 3 min). Two hundred fifty microliters of the organic phase was transferred to a new cup, the solvent was evaporated to dryness and the residue was dissolved in 100 µL ethanol. Twenty microliters of this solution was injected on the HPLC column (Nucleosil 100 RP-18 10 µm, 250×4.6 mm, Macherey-Nagel) and elution was performed as described before.

Determination of the Potassium Concentration

The concentration of potassium in noni fruit juices were determined with a Thermo Elemental AAS Spectrometer (Solaar M), equipped with a flame detector and a GF-95 graphite furnace. The flame detection technique with a potassium hollow cathode lamp was used for the investigation.

Sample Preparation for AAS Analysis

Commercial and self-prepared noni juices were centrifuged and diluted with distilled water (1: 10,000). This solution was directly supplied to the AAS. The AAS is equipped with quantitating software, which calculates the potassium ion concentration in milligrams per liter.

Preparation of a "Noni Juice" from Freeze Dried Noni Fruit Puree

For investigation of the evaporation rate of DTA during heat concentration, a noni juice was produced by dissolution of freeze-dried noni puree (Tahitian Noni International) in distilled water with dry matter concentration of 6%. This is equivalent to a natural noni juice. After filtration, 1 mL of the clear solution was heated at 95°C in a heating block, until 75% of the water was evaporated. The remaining solution was completed to 1 mL by addition of distilled water. This solution was extracted with EtOAc for HPLC analysis as described under the "HPLC Method for the Quality Assurance of Noni Juices" section.

Quantification of Organic Marker Substances

Quantification of the organic marker compounds, DTA and scopoletin, was performed by analysis of HPLC-chromatograms using the external standard method (ChromQuest). Stock solutions of pure compounds (1 mg/mL) were prepared in ethanol and a calibration curve with three concentrations each (0.1, 1.0, and 10 µg in 10 µL ethanol) was injected into the column for the calculation of the concentration/area ratio. Wavelengths of 346 and 254 nm were used for the determination of scopoletin and DTA, respectively. Each standard solution was measured in five replicates. The results showed an acceptable linearity.

Determination of the recovery rate was performed on a reconstituted noni juice, which was prepared from freeze dried noni puree as described under the "Preparation of a "Noni Juice" from Freeze Dried Noni Fruit Puree" section. This juice was extracted with EtOAc as described before. Another sample of this juice was extracted after addition of pure scopoletin and DTA (500 µg/mL). All analyses were performed in five replicates. The recovery rate for the marker compounds was found to be >90%. Due to this high recovery rate, no correction of the values was performed.

Results and Discussion

The need for a suitable analytical method for the quality assurance of commercial noni juices is evident. The price for such products is usually high and some manufacturers praise their products as "100% pure noni juice," "free of preservatives," "pure dripping juice," etc. However, the consumer has no ability to prove these statements. Because of the lack of an official analytical method for the quality assurance of noni juice products, governmental institutions usually do not routinely investigate such products. In the present investigation, we therefore provide an analytical method for the quality assurance of noni juice products, which is easy to perform and relatively inexpensive. It is based on two organic and one inorganic marker compounds.

The compound DTA is identified in *M. citrifolia*, and in the Rubiacea family for the first time. The compound has only been observed in the latex of *Euphorbia pulcherrima* Willd. (Warnaar 1977), and as a metabolite in the gram-positive bacterium *Streptomyces viridochromogenes* Tü 6105 (Armin et al. 1999) so far. In the latter study, the compound was also reported to have herbicidal activity. Because *Streptomyces* spp. live in soil, the herbicidal effect of the compound seems to be a defense mechanism against substrate competitors. The occurrence of DTA in the latex of a *Euphorbia* spp. indicates this compound may possibly also function as a repellent. The spectroscopic characteristics of the compound are shown in Fig. 1 and Table 1. Two isoforms of decadienal have been observed in avocado (*Persea amerciana*); however, neither the corresponding

acids nor decatrienal or decatrienoic acid was present in this fruit (Gaydou et al. 1987; Sinyida and Gramshaw 1998).

Polyunsaturated fatty acids (PUFAs) are present in food derived from plants and animals. The chain length of most of these compounds is 18–20 carbons, and the double bonds are mainly in the *cis*-position. With respect to this fact, DTA is not a common PUFA. These compounds are also substrates for lipoxygenases (LO), which are widespread in plant and animal tissues. In plants, oxidation of PUFAs by LO is involved in off flavors and post-harvest deterioration (Zhuang et al. 1994). It has been demonstrated that the corresponding aldehyde to DTA is present in fish oils (Ke et al. 1975). In the plant kingdom, DTA is rare, as mentioned before. We investigated a variety of tropical fruits (listed under the "Plant materials" section) with the analytical method described above and could not detect DTA in any of it (data not shown). The compound was therefore considered to be highly indicative of noni fruits. The second organic marker compound, scopoletin, is probably important for the anti-inflammatory properties of noni juice (Kim et al. 2004; Yu et al. 2008; Ding et al. 2008). This compound is not common to most fruit juices but occurs in several plants. The combination of DTA and scopoletin is therefore ideal to characterize the identity of noni juices (see Fig. 2).

Potassium is the most frequent mineral content in plant materials. Fruit juices normally contain between 1,000 and 3,000 mg/L (Belitz et al. 2008). Potassium contents of less than 1,000 mg/L normally indicate a dilution of a particular fruit juice with water. The average potassium content of 32 authentic noni fruit juices used as reference for this study was 2,245±1,196 mg/L.

In order to prove the quality and manufacturing processes of commercial noni juices on behalf of the three marker compounds, we first investigated the concentration of all three compounds in 32 samples of pure authentic noni juices, which were prepared from noni fruits harvested by us on 14 different islands in the Pacific and Indian Ocean. For the determination of the organic marker compounds DTA and scopoletin, we used an analysis based on HPLC separation described above. The same method was also used for the analysis of commercial noni fruit juice products. The results of the determination of scopoletin and DTA in these reference samples are shown in Table 2. As can be seen in this table, fresh noni fruits contain only small amounts of free DTA. The hydrolysis experiments of fresh squeezed juices clearly demonstrated that the majority of DTA is present in the glycoside form. This is demonstrated in the HPLC chromatograms of an untreated and hydrolyzed authentic noni fruit juice shown in Fig. 3.

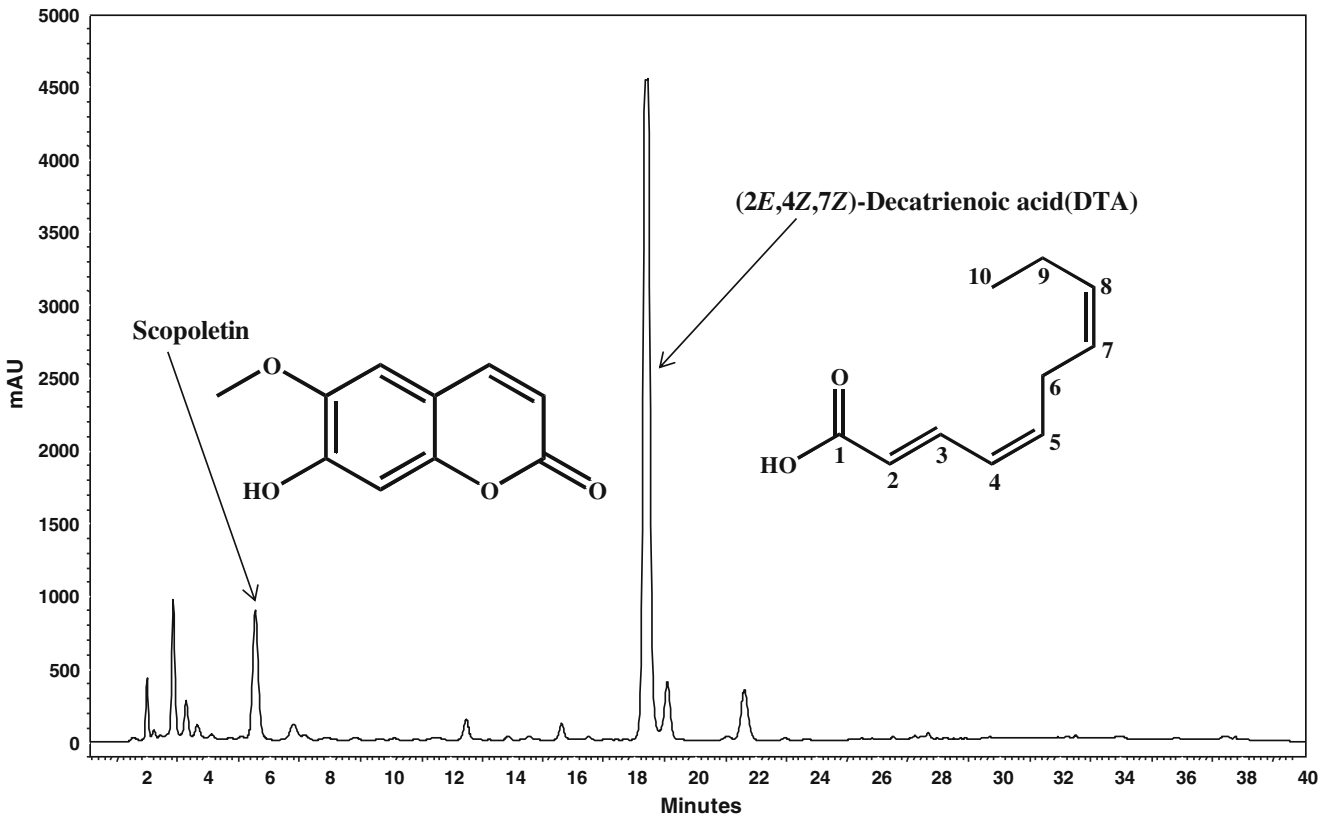

Fig. 2 HPLC chromatogram of an *n*-hexane extract of noni puree on Nucleosil RP-18 column

Table 2 Comparison of commercial noni juices (S1–S16) to the average of 32 pure self prepared noni juices (STD) with respect to three marker compounds: scopoletin, DTA and potassium

Sample	Scopoletin (μg/mL)	DTA (μg/mL)	DTA(A)[a]/DTA (G)[b]	Scopoletin/DTA ratio	K (mg/mL)	Dry weight (mg/mL)
S1	233±9	129±2	0.34	1.82	2.7	70.2
S2	25±3	35±5	0.11	0.70	0.4	128.0
S3	16±1	37±3	0.29	0.42	0.35	28.3
S4	174±8	105±6	0.44	1.66	2.7	54.1
S5	131±11	44±3	0.41	2.96	2.7	44.7
S6	20±2	29±4	0.20	0.69	0.25	70.7
S7	41±8	16±5	1.0	2.62	3.0	69.2
S8	113±15	24±1	1.0	4.66	3.4	86.0
S9	77±2	155±7	0.61	0.50	1.4	119.4
S10	9±3	15±3	0.21	0.65	0.4	23.9
S11	235±29	155±16	0.29	1.51	3.45	76.0
S12	150±7	79±10	0.29	1.91	1.6	141.9
S13	22±2	48±5	0.27	0.46	0.4	106.9
S14	26±2	58±6	0.70	0.45	0.6	69.8
S15	132±7	94±6	1.0	1.41	2.0	63.4
S16	75±2	300±29	1.0	0.25	1.0	147
STD	114±64	492±115	0.067	0.18	2.3±1.2	60±14

(A)[a] = aglycone, (G)[b] = Glycoside

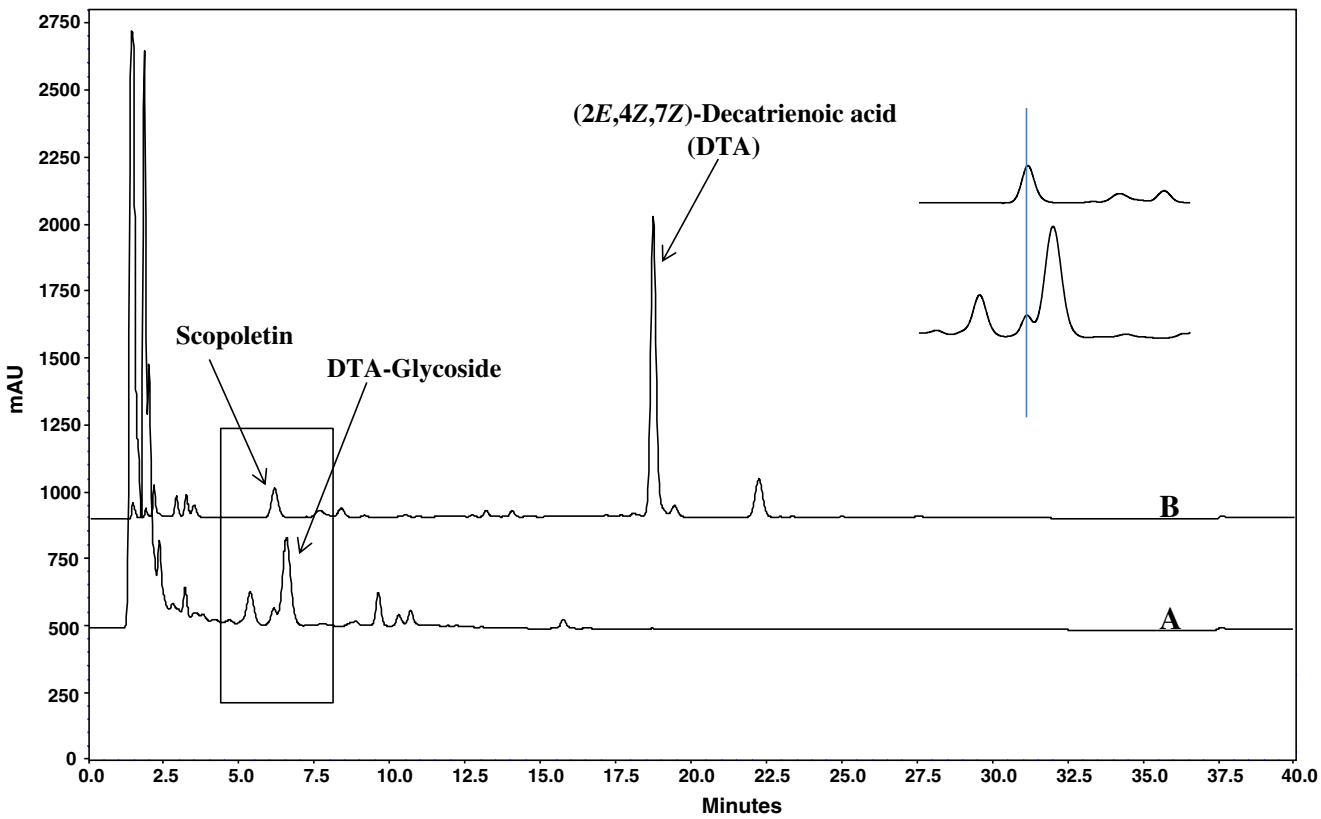

Fig. 3 **A** HPLC chromatogram of a pure noni juice, direct injection. **B** HPLC chromatogram of the same pure noni juice; injection after hydrolysis

The average relative concentration of free DTA in the 32 noni fruit reference samples was 8.3%. In contrast, scopoletin was almost completely present as aglycone in these samples.

Noni fruit juices on the market are mostly pasteurized. In the European Union, this is a prerequisite for marketing of noni fruit juice under the novel food regulation (European 2003). In order to investigate a possible cleavage of the glycosides of DTA by pasteurization, a sample of pure noni juice was heated for 20 min at 95°C. No change in the ratio of free to glycosidic bound DTA could be observed after analysis of the heated samples of noni fruit juice by HPLC (data not shown). The loss of glycosides of DTA in commercial noni fruit juices should therefore be a result of enzymatic degradation, for example, by a fermentation process.

Some manufacturers prepare their noni fruit juices from concentrates in order to keep the costs low for transport of the raw material. However, preparation by rehydration of a noni fruit juice concentrate was not indicated on the label of any of 16 commercial noni fruit juices investigated in this study. Heat concentration or freeze drying can both be used for the production of juice concentrates. For economic reasons, heat concentration is preferred in most cases. We therefore investigated the influence of heat concentration on the ratio of the partially volatile marker compound DTA to the non-volatile marker compound scopoletin. As seen from Fig. 4, the heat concentration has a considerable influence on the ratio of scopoletin/DTA. Before heating, the ratio was 0.373, and after heating, it increased to 1.4.

A summary of the concentrations of the three marker compounds investigated in 16 commercial noni fruit juice products and the average of 32 authentic untreated noni fruit juice samples is shown in Table 2. The relative concentrations with respect to the reference samples are also demonstrated in Fig. 5. The commercial juices S2, S3, S6, S10, S13, and S14 are below the 25% line of the reference samples with respect to all three parameters. There is no doubt that these juices are heavily diluted. Nevertheless, some of these juices are labeled as "100% pure noni juice." This is a misleading of the consumer. We could additionally detect benzoic acid in three of these juices (S2, S3, and S10) (data not shown). None of these juices indicated the addition of benzoic acid on the label, although this is a requirement by law. Noni juice does naturally contain very small amounts of benzoic acids (Farine et al. 1996); however, the concentrations detected in these juices exceeded the natural concentrations by more than 100-fold.

The specific noni fruit juice marker DTA was observable in all commercial samples investigated; however, only one of these juices (S16) contained more than 50% DTA compared to the reference juices, and three other juices (S1, S9, and S11) contained 25% or more. Further information comes from the scopoletin/DTA ratio and the

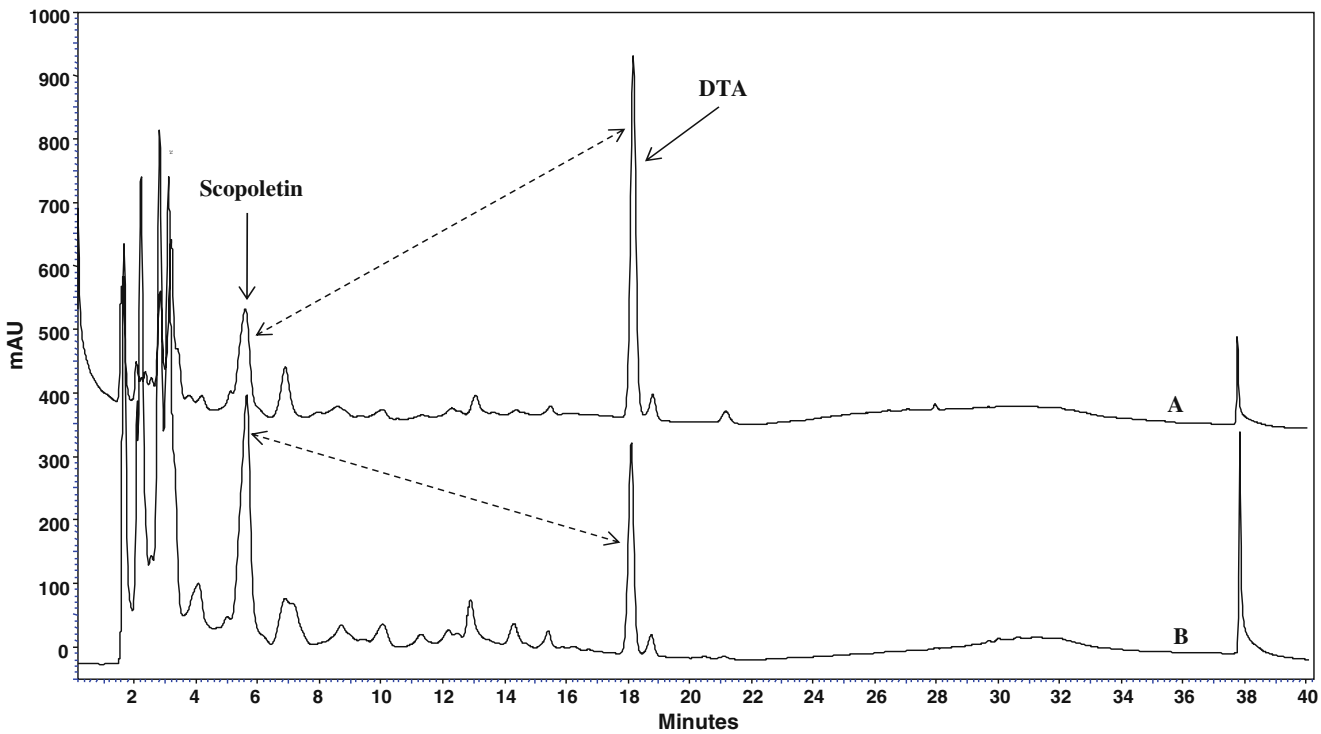

Fig. 4 The effect of heat concentration on pure noni juice. **A** HPLC chromatogram of a noni juice prepared from freeze dried noni fruit puree by rehydration. **B** HPLC chromatogram of the same noni juice after removal of 75% water at 95°C and rehydration to the original concentration

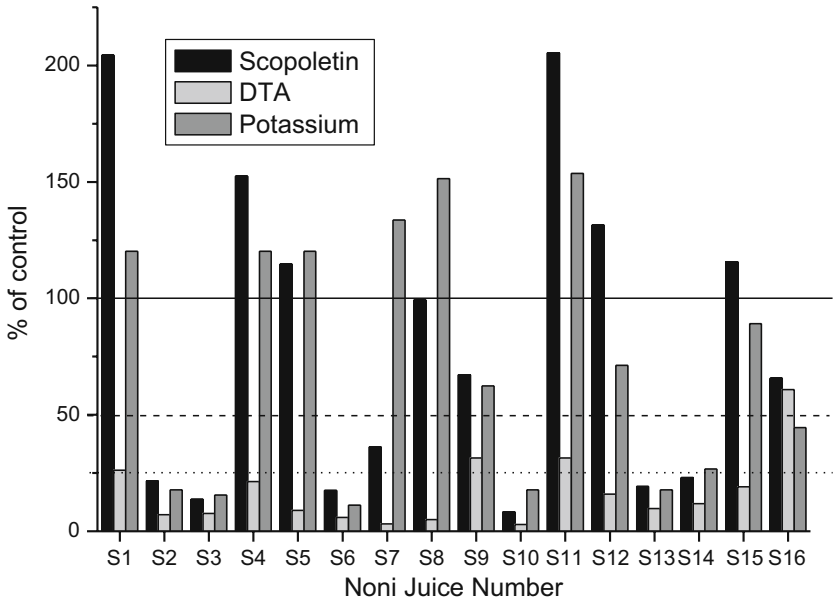

Fig. 5 Relative concentration of three marker compounds (scopoletin, DTA, and potassium) in 16 commercial noni fruit juices with respect to the average of 32 self prepared authentic noni fruit juices (control)

degree of DTA hydrolysation (Figs. 6 and 7). As outlined above and proven by an experiment in this study, heat concentration increases the relative concentration of scopoletin compared to DTA. Eight commercial juices (S1, S4, S5, S7, S8, S11, S12, and S15) showed scopoletin/DTA ratios comparable to (or higher than) our self-concentrated noni juice. We therefore suggest that these juices were prepared by rehydration of noni juice concentrates.

As outlined above, the degree of DTA glycoside hydrolysation is indicative for a possible fermentation of the noni fruit puree after harvest of the fruits. Freshly squeezed noni fruit juice does contain only very small amounts of free DTA, as seen in Fig. 7. Four commercial juices contained no DTA glycosides at all (S7, S8, S15, and S16), indicating that enzymatic processes sufficiently cleaved all of the glycosides. The juices S9 and S14 showed more than 50% and S1, S4 and S5 more than 30% degradation of DTA glycoside. The other juices (S2, S6, S10, S11, S12, and S13) showed DTA glycoside hydrolysation rates below 30%, indicating a limited enzymatic degradation. This could either be the result of an early heating or cooling step after squeezing the juices out of the fruits, which inactivates the enzymes. Interestingly, two of these juices (S11 and S12) were also among the candidates for a possible heat concentration, indicated by a high scopoletin/DTA ratio.

The dry average weight of our pure dripping juices was 59.6±13.8 mg/mL. Most of the commercial juices exceeded

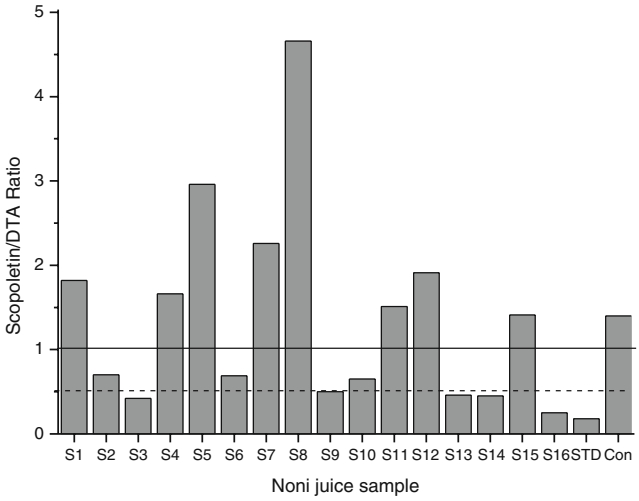

Fig. 6 Scopoletin-to-DTA ratio in commercial noni juices in comparison to the average of 32 self prepared pure noni juices (STD) and a noni juice heat concentrate (Con)

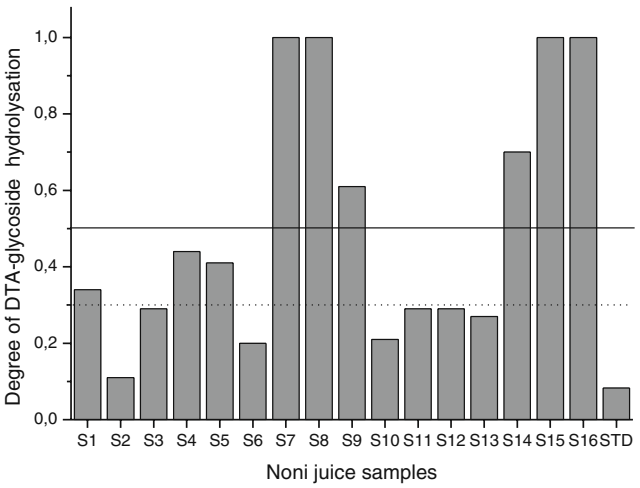

Fig. 7 Degree of DTA-glycoside hydrolysis in commercial noni juices in comparison to the average value of 32 self prepared pure noni juices

this value, except S3, S4, and S10. These juices have already been detected as diluted by the concentrations of the three marker compounds. Therefore, the dry weight values confirm our previous hypothesis. If the dry matter of the juices originates only from the noni fruit juice, it should correlate with the potassium concentrations. This is, however, not the case. Some juices contain pulp material, which adds to the dry weight but not the potassium amount, and others contain additives, such as preservatives (sodium benzoate) or fragrances, such as raspberry ketone (4-hydroxphenylbutylketone), which was observed in juices S2, S3, S6, S13, and S14 (data not shown).

Conclusion

Our investigation demonstrates that quality control of noni fruit juices occurring on the market is urgently needed. The method provided in this investigation serves as a suitable and inexpensive procedure for a screening of noni fruit juices, especially for governmental institutions. With only three marker compounds, it is possible to detect dilution, fermentation, and heat concentration of noni juices simultaneously.

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