Authentication of Noni *(Morinda citrifolia)* Juice

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Summary

The Noni plant *Morinda citrifolia* L. (Rubiaceae) is sold for various conjectural nutritional and health benefits. Methods using high-performance thin-layer chromatography (HPTLC) and headspace-solid-phase microextraction/gas chromatography/mass spectrometry (HS-SPME/GC/MS) to assess the authenticity of commercial Noni juices were developed in this study, allowing differentiating between authentic and adulterated products by use of multivariate data analysis (Principal Component Analysis). Parallel to the authenticity control, the HS-SPME method employed in this paper allowed detecting the preservatives benzoic acid and sorbic acid. In comparison to standard wet-chemical determinations, the chromatographic methods are more specific for Noni and also correspond to the aroma and flavour of the fruit.

Zusammenfassung

Die Noni-Pflanze *Morinda citrifolia* L. (Rubiaceae) wird aufgrund vermeintlicher ernährungsphysiologischer und gesundheitlicher Wirkungen in den Verkehr gebracht.

Zur Authentizitätskontrolle kommerzieller Noni-Säfte wurden in dieser Arbeit Hochleistungs-Dünnschichtchromatographie (HPTLC) und Dampfraum-Festphasenmikroextraktion/Gaschromatographie/Massenspektrometrie (HS-SPME/GC/MS) eingesetzt. Multivariate Datenanalyse (Hauptkomponentenanalyse) erlaubte dabei die Unterscheidung authentischer und verfälschter Produkte. Gleichzeitig mit der Authentizitätskontrolle konnten auch die Konservierungsstoffe Benzoesäure und Sorbinsäure mittels HS-SPME bestimmt werden. Im Vergleich mit den üblichen nasschemischen Bestimmungsmethoden erlaubten die vorgestellten chromatographischen Methoden einen spezifischeren Nachweis von Noni und die Ergebnisse korrespondieren mit dem fruchttypischen Aroma und Geschmack.

Keywords: Noni, *Morinda citrifolia* L. (Rubiaceae), juice, authenticity control, food legislation / Noni, *Morinda citrifolia* L. (Rubiaceae), Säfte, Authentizitätskontrolle, Lebensmittelrecht

1 Introduction

Morinda citrifolia L. (Rubiaceae), commonly known as "Noni", but also known as "Indian mulberry" and "Nonu", is a shrub, which grows in sandy areas along many tropical coasts, and is typically found in the Hawaiian and Tahitian islands. Its "grenade-like" shaped fruits are green until maturity, when they rapidly turn to a light yellow and then a translucent white. The fruit has an unsound taste and a soapy smell being ripe. Noni is sold for various nutritional and health benefits, which are mostly anecdotal with limited scientific evidence[1)]. Especially on the Internet, the commercially available Noni preparations are recommended for almost all health problems including cancer, diabetes, AIDS, digestive and bowel conditions, high blood pressure, menstrual cramps, viral, bacterial and helminthic infections, rheumatic diseases, mental depression, senility, pain, chronic fatigue syndrome and other chronic disorders[1,2)]. Isolated cases of acute hepatotoxicity[3,4)] as well as renal insufficiency leading to hyperkalemia[5)] have been reported after ingesting Noni. Recently, it was emphasised that Noni juice should be avoided in the dialysis population because of its high potassium content[6)].

The European Union (EU) banned the marketing of Noni in the late 1990s because of unknown safety, which started a controversial discussion[7)] whether products made of Noni should be considered as a Novel Food according to European Regulation No 258/97[8)]. Meanwhile, the Scientific Committe on Food (SCF) has considered Tahitian Noni juice, at the observed levels of intake, as being acceptable. Therefore, on the 5th June 2003, the European Commission authorised the placing on the market of a Noni juice as a novel food ingredient for use in pasteurised fruit drinks[9)]. Other companies may sell Noni based products, if they can demonstrate that their products are "substantially equivalent" to those already on the market. The term "Noni juice" or "juice of Morinda citrifolia" must be displayed on the labelling of the products or in the list of ingredients. Nowadays, a large variety of Noni products is available on the market.

For authenticity and quality control of Noni juice, no methods are currently available. But an interesting approach is the analysis of volatile compounds. Previous studies revealed the presence of approx. 50 detectable volatile compounds in ripe Noni fruit. The most abundant compounds are octanoic and hexanoic acids, as well as their corresponding methyl and ethyl esters, which are also the major contributors to the soapy aroma of the ripe Noni fruit[10,11)]. In the present study, high-performance thin-layer chromatography (HPTLC) as well as the combination of headspace

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solid-phase microextraction (HS-SPME) and gas chromatography with mass spectrometry (GC/MS)[12–14] is used for the first time to evaluate commercial Noni juices. The analysis results are compared using multivariate data analysis (Principal Component Analysis).

2 Experimental

2.1 Reagents and materials

Cyclodecanone, used as internal standard for HS-SPME/GC/MS, was purchased as a solid from *Fluka* (Buchs, Switzerland). It was stored at 8 °C, and used after dilution to the required concentrations. Further chemicals were purchased from *Merck* (Darmstadt, Germany). An SPME device for the autosampler with a replaceable 100 µm polydimethylsiloxane (PDMS) fibre was obtained from *Supelco* (Deisenhofen, Germany). The fibre was conditioned at 250 °C for 1 h in the injection port of the GC according to the supplier's instructions.

Noni beverages (n = 22) were collected in the context of the official food control in the German Federal State of Baden-Württemberg.

2.2 HPTLC method

Classic thin-layer chromatographic methods are suitable to investigate the authenticity of Noni beverages. Separation was performed on pre-coated 10 x 10 cm HPTLC glass plates (sorbent: silica gel; pore size: 60 Å; fluorescence indicator: F254; *Merck*, Darmstadt, Germany). Sample volumes of 20 µl were applied to the plates as bands with a width of 8 mm using a TLC applicator (Automatic TLC Sampler III, *CAMAG*, Berlin, Germany). For development and visualisation, two different methods were suitable for Noni. Method 1 was a modified assay for anthracene derivatives by *Stahl*[15]. Method 2 was modified from an assay for hydroxyanthracen glycosides given in the European Pharmacopoeia[16]. The conditions are detailed in Table 1.

Following chromatographic separation bands can be compared to Noni-typical bands of authentic comparison samples and authenticity of the sample can be evaluated.

Tab. 1 HPTLC conditions

	Method 1 (Multiple development)	Method 2
Mobile phase	1st step: Ethyl acetate/methanol/water (10/1.7/1.3, v/v/v), distance 4 cm 2nd step: toluene/ethyl formiate/formic acid (7.5/2.4/0.1, v/v/v), distance 8 cm	Acetic acid/water/ethyl acetate/propanol-1 (1/3/4/4, v/v/v/v)
Staining reagent	Ammonia vapour	Nitric acid (20%, v/v), followed by heating for 10 min at 120°C
Visualisation	UV, 365 nm	UV, 365 nm

2.3 HS-SPME/GC/MS method

The HS-SPME/GC/MS procedure was modified from a previously published method for the quality control of Aloe vera beverages[17].

1 ml of the Noni sample was submitted into a 10 ml headspace vial in the presence of 50 µl cyclodecanone (50 ng/ml). No further sample preparation was necessary. The vial was sealed using a silicone septum and a magnetic cap and was shaken for 5 min at 40 °C in the agitator of the autosampler (650 rpm, agitator on time: 0:05 min, agitator off time: 0:02 min). For absorption the needle of the SPME device containing the extraction fibre was exposed into the headspace of the vial for 11 min. The compounds absorbed on the fibre were desorbed by exposing the fibre in the injection port for 5 min and then analysed.

The analyses were performed on a model 6890 Series Plus gas chromatograph, in combination with an Agilent 5973 N MSD mass spectrometer (*Chromtech*, Idstein, Germany). Substances were separated on a fused silica capillary column (HP-5MS, 30 m x 0.25 mm I.D., film thickness 0.25 µm). The temperature programs were applied as follows: start temperature 35 °C, 10 °C/min increase up to 300 °C. The temperatures for the injection port, ion source, quadrupole and interface were set at 260 °C, 230 °C, 150 °C and 280 °C, respectively. Injection was carried out in splitless injection mode, and helium at a flow rate of 1.0 ml/min was used as carrier gas. Electron impact (EI) mass spectra for the analytes were recorded in Full Scan mode.

2.4 Multivariate data analysis

The quantitative data of HS-SPME/GC/MS analyses were exported to the software Unscrambler v9.2 (*CAMO Process AS*, Oslo, Norway). The data set was pre-processed by standardization to give all variables the same variance. Then Principal Component Analysis (PCA) was used to transform the original measurement variables into new variables called principal components (PC). The technique of cross-validation was applied to determine the number of principal components (PCs) needed. During cross-validation, one sample at a time (of n samples) is left out, and the prediction ability is tested on the sample omitted. This procedure is repeated n times resulting in n models and will give an estimate on the average prediction ability for the n models. This result is used to select the number of PCs needed. By plotting the data in a coordinate system defined by the two largest principal components, it is possible to identify key relationships in the data as well as to find similarities and differences.

3 Results and Discussion

Thin-layer-chromatography is a simple and rapid method to detect adulterations

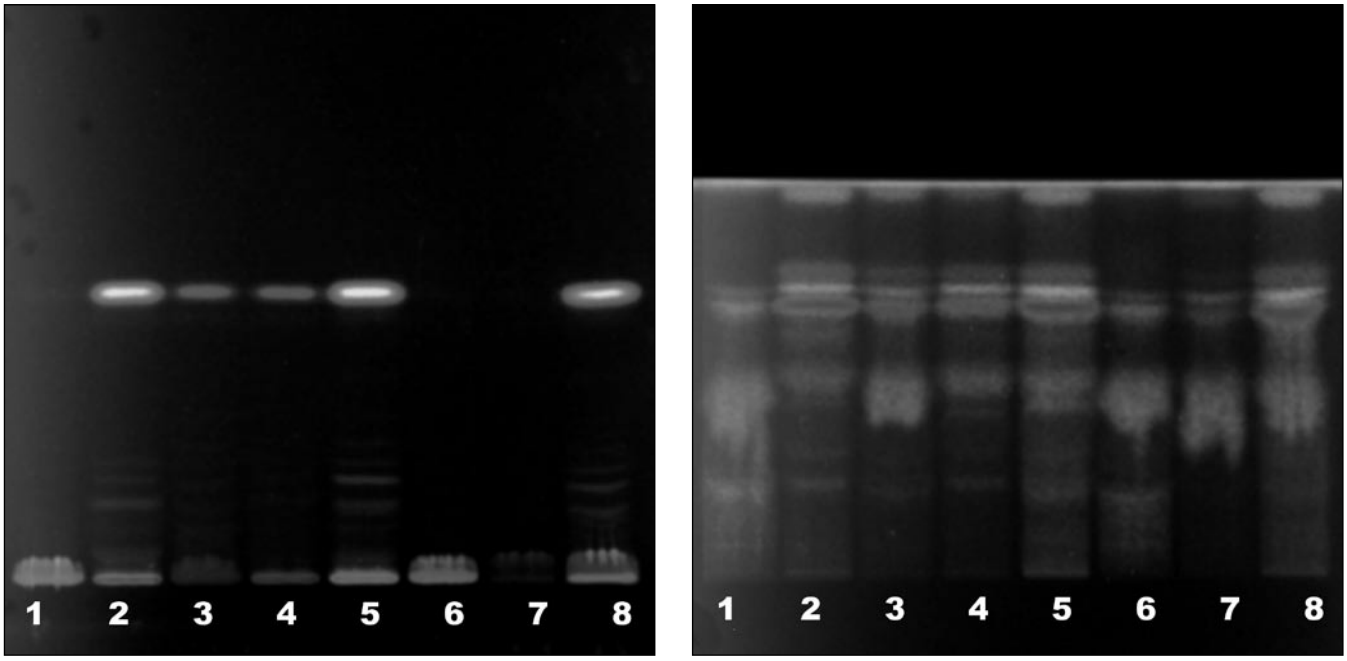

Fig. 1 HPTLC chromatograms in fluorescence scan of Noni juices developed with method 1 (left) and method 2 (right) (**2,5:** authentic juice; **3,4,6,8:** commercial juice; **1,7:** Noni (< 10 %) mixed with other juices)

of Noni products. Typical representations of the developed plates are shown in Figure 1. Samples with none or only low contents of Noni can be easily distinguished from 100 % Noni samples.

In comparison to HPTLC, HS-SPME/GC/MS-chromatograms contain considerably more data and may, therefore, be used to confirm the preliminary results of HPTLC. A typical HS-SPME/GC/MS chromatogram of a Noni juice is shown in Figure 2. The new HS-SPME procedure seems to be suitable for the determination of volatile compounds of Noni in food products in an automated and, therefore, convenient procedure. All steps (e.g. heating and shaking of the sample, absorption, pre-concentration and desorption into the injector of the GC) are programmable and are automatically executed, meaning that the number of sources of error is reduced distinctly concerning the reproducibility. A large advantage of the HS technique in relation to the direct immersion is the protection of the SPME fibre and the significant reduction of matrix effects in chromatography.

The abundance of data in the HS-SPME/GC/MS-chromatograms (over 30 substances were identified and quantitated) can be made accessible using multivariate data analysis.

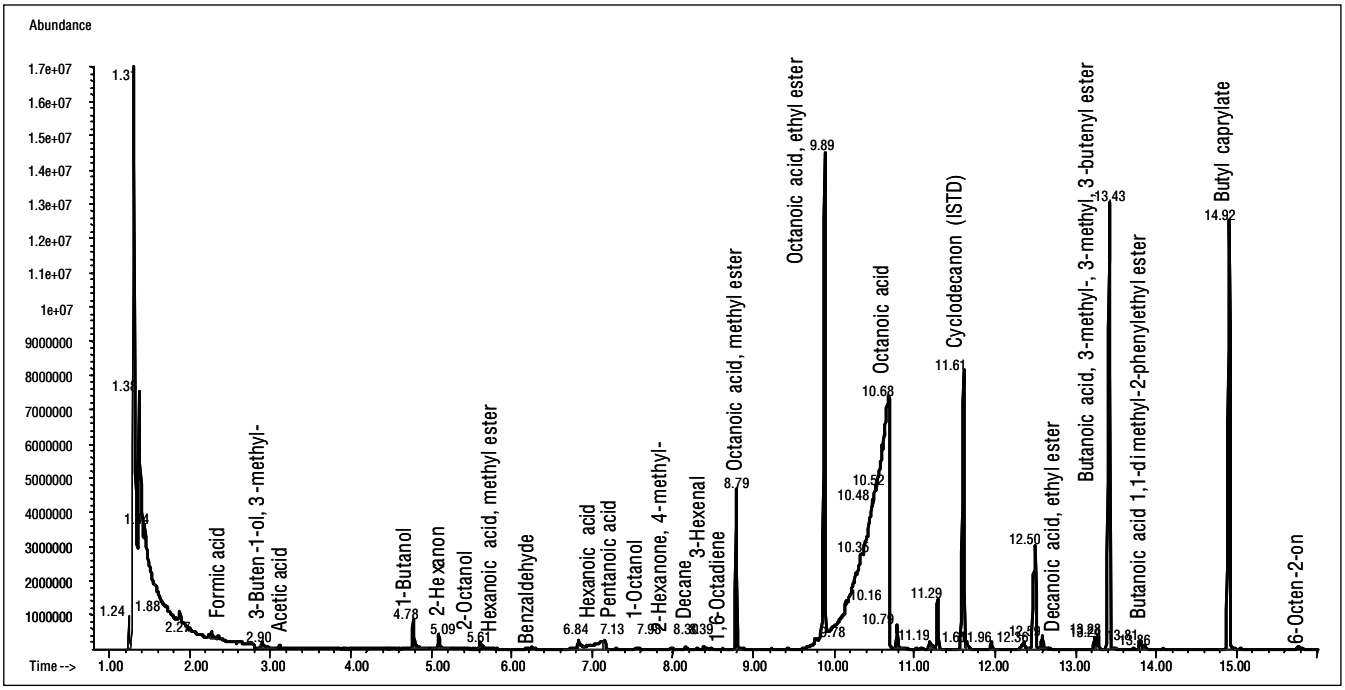

Fig. 2 HS-SPME/GC/MS Chromatogramm of an authentic Noni juice

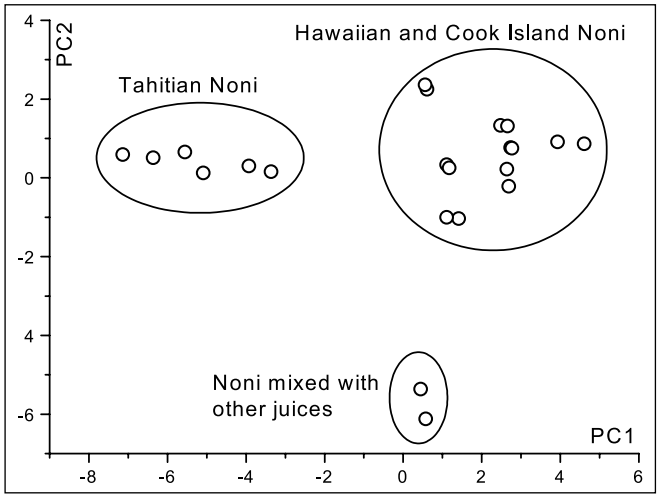

Fig. 3 PCA Scores Plot of the HS-SPME/GC/MS analysis results

The scores scatter plot of the first two PCs calculated using all integrated peaks is shown in Figure 3. Three groups of samples are noted. Tahitian Noni cluster in a range lying toward the negative side of PC1. Whereas the scores near zero or in positive PC1 corresponding to Hawaiian or Cook Island juices. A third group with negative PC2 values could be attributed to mixed juices with low quantities of Noni. Because of the commercial character of our samples, it could not be determined if the separation was due to geographic differences arising e.g. from climatic differences or different plant varieties, or only different processing methods (traditional method or concentration using freeze-drying). Further authentic samples with known processing conditions would be required to assess our technique as suitable to determine the origin of Noni.

From the PCA-Loadings Plot, the following substances with the greatest influence on the model were extracted, which can be seen as suitable markers for Noni authenticity: pentanoic acid, hexanoic acid and octanoic acid, as well as their ethyl esters. Therefore, our study confirms the first results of other working groups[10,11].

Simultaneously with the authenticity control, the HS-SPME method employed in this work allowed the determination of the food preservatives benzoic acid and sorbic acid. Benzoic acid was found in one sample without approval or even labelling. Another problem in the labelling of Noni juice is the illegal advertisements with reference to scientifically not proven health benefits.

4 Conclusions

HPTLC and HS-SPME/GC/MS might also be usable to determine if Noni juices are substantially equivalent to existing foods as required in the EU novel food regulation[8]. So far, only very unspecific parameters like water content, total protein, ash, fat, carbohydrates, density, or pH value were used to determine the equivalency. The pattern of anthracene derivatives ("HPTLC fingerprint") or of volatile components ("HS-SPME/GC/MS fingerprint") are more specific for Noni and also correspond to the aroma and flavour of the fruit. Both methods are also faster than the standard wet-chemical determinations, and require less amounts of sample and solvents.

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