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Light-induced alterations of pineapple (*Ananas comosus* [L.] Merr.) juice volatiles during accelerated ageing and mass spectrometric studies into their precursors

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ABSTRACT

Alterations of volatiles during accelerated light-induced ageing of pineapple juice were assessed by HS-SPME-GC–MS in a non-targeted profiling analysis over a 16-week period. Multivariate statistics permitted to reveal substantial chemical markers generally describing the effect of light storage. Volatiles generated comprised phenylpropenes, carbonyls, 2-methylthiophene, toluene, and furfural, while concentrations of methyl and ethyl esters, terpenes, and furanones decreased. In addition, the qualitative composition of phenolic compounds and glycoside-bound volatiles in selected samples was characterized by HPLC-DAD-ESI-MS^n as well as HR-ESI-MS. The fresh juice contained unique pineapple metabolites such as *S*-*p*-coumaryl, *S*-coniferyl, *S*-sinapylglutathione, and structurally related derivatives. Among others, the presence of *p*-coumaroyl, feruloyl, and caffeoylisocitrate as well as three 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone glycosides in pineapples could be substantiated by the HR-ESI-MS experiment. Mass spectrometric assignments of selected metabolites are presented, and putative linkages between volatiles and their precursors are established.

1. Introduction

Many consumers appreciate juices from tropical fruits owing to their unique exotic flavor. Particularly, during storage at retail and end-consumer households, beverages may be exposed to light and elevated temperatures. As a consequence, alterations in the chemical composition are to be expected, and the sensory juice quality may be adversely affected.

Light-induced alterations of volatiles have been characterized in milk (Borle, Sieber, & Bosset, 2001) and alcoholic beverages such as beer and wine (Reineccius, 2006). By contrast, fruit juices have only been investigated to a limited extent. Several off-flavor compounds deriving from the oxidation of polyunsaturated fatty acids like ketones and aldehydes evolved in turbid apple juice stored under fluorescent light (Hashizume, Gordon, & Mottram, 2007; Hashizume, Okugawa, Gordon, & Mottram, 2006). An increase of terpene alcohols, furans, and volatile phenols has been observed during storage of orange juice. Furfural has been proposed as an indicator for prolonged storage time, while α-terpineol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), and 4-vinylguajacol evoked an off-odor in aged orange juices (see review by Perez-Cacho and Rouseff (2008)). In pineapple juice, a “cabbage-like” off-flavor emerging during storage has been reported in the literature. This light-induced off-flavor has been attributed to methional as well as dimethyl di-, tri-, and tetrasulfide (Baumann, Maier, & Gierschner, 1992). The aforementioned sulfurous compounds derive from Strecker degradation of methionine during thermal treatment or from a riboflavin-sensitized photoreaction (Baumann et al., 1992; Borle et al., 2001). Similarly, Steinhaus, Thomas, and Schieberle (2014) have proposed methional as causing a “cooked vegetable-like” off-flavor in pasteurized pineapple juice. However, further volatiles are assumed to contribute to the off-flavor in aged pineapple juice so far lacking detailed investigations.

Consequently, the present study aimed at an in-depth characterization of pineapple juice volatiles during accelerated ageing under illumination. A non-targeted headspace solid phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS) profiling analysis should reveal substantial chemical markers describing the

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Abbreviations: CID, collision-induced dissociation; E(Z), pyroglutamic acid; G, glycine; GC–MS, gas chromatography–mass spectrometry; GSH, glutathione; HDMF, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone; HPLC-DAD, high performance liquid chromatography-diode array detection; (HR)-ESI-MS, (high-resolution) electrospray ionization mass spectrometry; HS-SPME, headspace solid phase microextraction; λ_{max}, UV maxima; LRI, linear retention index; LV, latent variable; MDMF, 4-methoxy-2,5-dimethyl-3(2*H*)-furanone; n.d., not detected; sh, shoulder; SPE, solid phase extraction; t_R, retention time; VID, variable identification (coefficient); wap, weeks after processing

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alterations during storage. Complementary, high performance liquid chromatography-diode array detection-electrospray ionization multiple-stage mass spectrometry (HPLC-DAD-ESI-MS^n) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis shall unravel putative non-volatile precursors, i.e., phenolic compounds and glycoside-bound volatiles. Finally, putative formation and degradation pathways of selected volatiles should be suggested.

2. Materials and methods

2.1. Pineapple juice samples

Sea-freighted MD2 (syn. "Extra Sweet") pineapples (*Ananas comosus* (L.) Merr.) from Cote d'Ivoire were purchased from a local fruit distributor (Schumacher, Filderstadt-Bernhausen, Germany). For postharvest flavor genesis, green-ripe pineapples were stored for one week at room temperature. Juice processing was performed at a pilot plant scale in three separate batches each using 24 individual fruits. After removing their crowns, pineapples were washed and manually peeled. Juice was extracted at 900 rpm using a food mill (model PAP 0533, Alberto Bertuzzi, Brugherio, Italy) equipped with a sieve of 1.6 mm mesh width followed by a second separation step with a 0.8 mm sieve. The raw juice obtained was homogenized using a high-pressure homogenizer (type LAB 60-10 TBSX, APV Gaulin, Lübeck, Germany) operated at 180 bar. Subsequently, 20 g/kg citrus pectin with a 47% degree of esterification (Herbstreith & Fox, Neuenbürg, Germany) was added for cloud stabilization. The juice was pasteurized at 90 °C using a pilot plant scale pasteurizer (Ruland Engineering & Consulting, Neustadt, Germany). Samples were filled in 0.1-L clear glass bottles using a Schmalbach-Lubeca (Braunschweig, Germany) filling and sealing machine, and immediately cooled to ~8 °C in a water bath.

For accelerated ageing over a total of 16 weeks, juice samples were stored in a climate cabinet (KBF-ICH 720, Binder GmbH, Tuttlingen, Germany) maintained at 25 °C and illuminated with eight fluorescent tubes (light color 640, cool white, 400–800 nm) and two UV-A tubes (light color 09, 320–400 nm). According to the manufacturer's specification, the resulting spectral distribution was in accordance with CPMP/ICH/279/95 (Q1B) option 2 (ICH, 1996). The samples were randomly rearranged once a week to compensate for possible variations in the light intensity and heating effects of the lamps. One bottle of each batch ($n = 3$) was analyzed 1, 2, 4, 8, 12, and 16 weeks after processing (wap) and compared to the freshly processed juice (0 wap). All samples were filled in 20-mL headspace vials and stored at −80 °C until analysis.

2.2. GC–MS analysis

A 7 ± 0.1 g juice sample was spiked with 100 μL of an aqueous solution containing 0.0824 g/L 2-methylpentan-1-ol as an internal standard. The resulting peak area of the internal standard ranged between those of volatiles featuring high (e.g., methyl hexanoate) or intermediate to low responses (e.g., ethyl acetate and HDMF) in the HS-SPME-GC–MS chromatograms. HS-SPME-GC–MS analyses were performed using an Agilent 6890 N gas chromatograph and an Agilent 5975 Mass Selective Detector (both Agilent Technologies, Santa Clara, CA, USA) as described previously (Steingass, Grauwet, & Carle, 2014). Briefly, volatiles were isolated by HS-SPME for 60 min at 40 °C using a 65 μm PDMS/DVB fiber (Supelco, Bellefonte, PA, USA). GC separation was achieved using a fused silica capillary column coated with polyethylene glycol (30 m × 0.25 mm, $d_f = 0.25$ μm, DB-Wax, Agilent J & W Columns, Santa Clara, CA, USA). Mass spectrometric detection was performed applying the positive electron impact (EI +) mode at 70 eV and a scan range of m/z 40–270. Individual volatiles were tentatively assigned by their mass spectra, linear retention indices (LRIs) (Akioka & Umano, 2008; NCBI, 2017; NIST, 2008), and the comparison with authentic reference standards.

2.3. HPLC-DAD-ESI-MS^n analysis

Juice samples were centrifuged for 10 min at 3500 rpm (Labofuge 200, Heraeus Material Technology, Hanau, Germany). 10 mL of the supernatant obtained was subjected to solid phase extraction (SPE) using a Chrombond® C18ec cartridge (Macherey-Nagel, Düren, Germany) according to the protocol established previously (Steingass, Glock, Schweiggert, & Carle, 2015).

HPLC-DAD-ESI-MS^n analyses were conducted applying an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) fitted with a RP-C18 analytical column (Kinetex™, 250 × 4.6 mm, 5 μm particle size, 100 Å pore size core-shell particles), and a C18 guard column (SecurityGuard™ ULTRA cartridge, both from Phenomenex, Aschaffenburg, Germany). Elution solvents, the gradient, and system settings were used as previously reported (Steingass, Glock, et al., 2015). Electrospray ionization (ESI) mass spectra were acquired at a scan range of m/z 50–800 using an Esquire 3000 + ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany).

2.4. HPLC-DAD-HR-ESI-MS analysis

HPLC-DAD-HR-ESI-MS analyses were performed using an Agilent 1290 UHPLC system interfaced with a Q Exactive Plus high-resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). HPLC conditions were set as detailed above. ESI mass spectra in the positive/negative ion mode were acquired applying the following conditions: scan range, m/z 150–1300; sheath gas flow, 20/60; auxiliary gas, 6/20; sweep gas flow, 0/5; spray voltage, 4.20/3.0 kV; capillary temperature, 320 °C; auxiliary gas heater, 320 °C. Data evaluation was performed with XCalibur software version 4.0 (Thermo Fisher).

2.5. Reagents and SPE cartridges

2-Methyl-1-pentanol (CAS 105-30-6), eugenol (CAS 97-53-0), 4-allyl-2,6-dimethoxyphenol (CAS 6627-88-9), 2-methylthiophene (CAS 554-14-3), HDMF (CAS 3658-77-3), and 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (MDMF, CAS 4077-47-8) were purchased from Sigma-Aldrich, isoeugenol (mixture of isomers, CAS 97-54-1) from Merck (Darmstadt, Germany). Toluene (CAS 108-88-3), methanol (CAS 67-56-1), and hydrochloric acid (CAS 7647-01-0) were from VWR International (Darmstadt, Germany), formic acid (CAS 64-18-6) from Merck. SPE cartridges were obtained from Macherey-Nagel (Düren, Germany). Double-distilled water prepared with an arium® 611 UV (Sartorius, Göttingen, Germany) ultrapure water system was used throughout.

2.6. Statistics

HS-SPME-GC–MS data obtained was subjected to partial least squares (PLS) regression using the concentration of the volatiles as X-variables and the weeks after processing (wap) as Y-variable. After calculation of variable identification (VID) coefficients, discriminative volatiles with VID $\geq |0.80|$ were assigned (Steingass, Jutzi, Müller, Carle, & Schmarr, 2015).

3. Results

3.1. HS-SPME-GC–MS profiling

A total of 166 peaks was detected in the HS-SPME-GC–MS total ion current chromatograms of the juice samples (see Fig. A.1). To identify most discriminative volatiles, a PLS regression was calculated. The latent variables LV1 (84.5%) and LV2 (12.0%) explained 96.5% of variance in the Y-variables. Thus, PLS regression clearly fitted an effect of storage further underlined by the length of the Y-loading vector. The corresponding biplot is illustrated by Fig. 1.

Most discriminative volatiles (VID $\geq |0.80|$) describing the changes