Authentication of pineapple (*Ananas comosus* [L.] Merr.) juice from pulp and peel by HPLC-DAD-(HR)-ESI-MS^n analysis

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Abstract

Large quantities of by-products, such as peels and trimmings, emerge during industrial pineapple (*Ananas comosus* [L.] Merr.) processing. The latter may be further exploited by juice extraction. However, the low-quality juices obtained may be marketed as genuine pineapple juice from pulp or be admixed to the latter, thus adulterating the final product. To identify chemical markers, juice was extracted from edible pulp, from flesh adhered to the peel, and from milled peel. The metabolite pattern in the juices was elucidated by HPLC-DAD-(HR)-ESI-MS^n. Unsupervised principal component analysis (PCA) and hierarchical cluster analysis (HCA) calculated on the basis of physico-chemical parameters and metabolite profiles enabled the distinction between juices from pulp and those from peel, irrespective of the fruit maturity degree. In addition, specific ratios of selected marker compounds were calculated, permitting the unambiguous distinction between pulp and peel juices as well as the three maturity stages assessed.

Keywords: adulteration; juice quality; phenolic compounds; furanones

Abbreviations: CID, collision-induced dissociation; *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 ionisation mass spectrometry; λ_{max}, UV maxima; *MDMF*, 4-methoxy-2,5-dimethyl-3(2*H*)-furanone; *n. d.*, not detected; *MW*, molecular weight; PCA, principal component analysis; *sh*, shoulder; t_R, retention time

1 Introduction

Pineapple (*Ananas comosus* [L.] Merr.) represents one of the leading fruits of the tropics worldwide. The major producing areas are Southeast Asia and Latin America. Considerable amounts of fresh fruit, juice, jam, jelly, and dried pineapple products are exported to North America and Europe (Li et al., 2014; Steingass, Glock, Schweiggert, & Carle, 2015; Mhatre, Tilak-Jain, De, & Devasagayam, 2009). Pineapples and derived products are popular owing to their pleasant aroma and flavour. They contain considerable concentrations of polyphenols, vitamins, and other compounds possibly exerting health benefits. Merely 60% of the pineapple infructescence is edible, thus processing residuals range between 45 and 65% (da Silva, Nogueira, Duzzioni, & Barrozo, 2013). The canning industry is producing large quantities of liquid and solid wastes *inter alia* peels and trimmings (Li et al., 2014). In order to further exploit these residues; processors also extract juice from the aforementioned by-products. The low-valued juice extracted from the peel or even the "mill juice", i.e., the liquid obtained from finely milled pineapple shell, is commonly used as syrup during canning. However, it may also be admixed to pineapple juice, thus adulterating the final product, or even be marketed as the genuine produce.

Adulteration of food, and especially beverages, is a serious issue in the global market. Fruit juices represent one of the most common targets for fraud (Jandrić et al., 2014). According to the Directive 2001/112/EC, fruit juice shall be exclusively obtained from the edible fraction of the fruits. Pineapple peel is considered as `not edible´, but is a good source for recovery of valuable compounds such as polyphenols. Peel-specific metabolites may provide a useful tool to reveal the fraudulent admixture of juice extracted from pineapple shell (Wen & Wrolstad, 2002; Fügel, Carle, & Schieber, 2005; Steingass et al., 2015a). Several analytical methods have been proposed to identify different types of adulterations in fruit juice. Techniques applied comprise profile analysis of sugars, organic acids or flavonoids, as well as analysis of minerals, trace metals, and stable isotopes using high performance liquid chromatography or gas chromatography (Ehling & Cole, 2011; Gómez-Ariza, Villegas-Portero, & Bernal-Daza, 2005; Muntean, 2010), capillary electrophoresis (Saavedra, Rupérez, & Barbas, 2001), ^{1}H NMR spectroscopy (Cuny et al., 2008), inductively coupled plasma mass spectrometry (Schwartz & Hecking, 1991), and neutron activation analysis (Anderson, Cunningham, & Alvarez, 1992). In most instances, the analytical tools are comparatively expensive. A metabolomics approach (Jandrić et al.,

2014) also in combination with chemometrics (Jandrić, & Cannavan, 2017) has been proposed as an economically reasonable alternative. However, also a LC-MS based profiling requires advanced analytical tools being unaffordable for, e.g., small juice processing companies.

In most instances, adulterations are difficult to uncover. However, in order to protect the consumers, it is essential to guarantee authenticity and compliance with the product specification. Moreover, from the economic point of view, product authentication is essential to avoid unfair competition that may even destabilise the market (Hong et al., 2017).

For these reasons, the aim of the present work was to establish an appropriate analytical tool to reveal the fraudulent usage of juice from pineapple peel. Specific patterns of metabolites that can be easily detected by HPLC-UV, comprising phenolic and amino compounds as well as furanones and their glycosides, were assessed in pineapple juices obtained from pulp and peel. To unravel a possible influence of fruit maturity, juices were produced from fresh and stored pineapples as well as fruits at the end of their commercial shelf-life. Compound identifications shall be substantiated by detailed HPLC-DAD-(HR)-ESI-MS^n analyses.

2 Materials and methods

2.1 Reagents

4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (MDMF), sinapyl alcohol, coniferyl alcohol, gallic acid, and tris(2-carboxyethyl)phosphine hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Caffeic, sinapic, *p*-coumaric, and ferulic acid were from Roth (Karlsruhe, Germany). Syringic acid was from Extrasynthese (Genay, France), L-tyrosine from Fluka Chemie (Buchs, Switzerland), and serotonin hydrochloride from Sigma-Aldrich. Methanol, L-ascorbic, hydrochloric, and meta-phosphoric acid were purchased from VWR International (Darmstadt, Germany). Formic acid, Folin-Ciocalteu reagent, formaldehyde solution (~37%), dipotassium hydrogen phosphate (K_2HPO_4), sodium carbonate (Na_2CO_3), and sodium hydroxide solution (0.25 N, Titripur®) were obtained from Merck (Darmstadt, Germany). Potassium hydrogen phthalate was purchased from Th. Geyer (Renningen, Germany). Double-distilled water (ddH_2O)

prepared with an arium® 611 UV (Sartorius, Göttingen, Germany) ultrapure water system was used throughout.

2.2 Pineapple juice samples

Air-freighted MD2 (syn. "Extra Sweet") pineapples (*Ananas comosus* (L.) Merr.) from Ghana were purchased from a local fruit distributor (Schumacher, Filderstadt-Bernhausen, Germany). The pineapples were stored for up to two weeks at a temperature of 10–13 °C and a relative humidity of 50–60%.

2.2.1 Laboratory scale production

Juice production was performed from fresh pineapples (T0) as well as after seven (T7) and fourteen days (T14) of storage, respectively. Five individual batches were produced each maturity stage. The juice was extracted from pulp with a food mill (Gastroback, Hollenstedt, Germany) and from peel and peel milled with a crusher (Bucher, Niederweningen, Switzerland), respectively, using a Hafico tincture press (Fischer Maschinenfabrik, Neuss, Germany). The pasteurisation was achieved at 90 °C for 5 min by incubating the samples filled in glass bottles in a water bath (Lauda; Lauda-Königshofen, Germany). Samples were immediately cooled in a water bath after processing.

2.2.2 Pilot plant production

Juice processing was additionally performed at a pilot plant scale using fruits stored for seven days (T7). After removing their crowns, pineapples were washed and manually peeled. For the pilot plant production, juice was extracted from pulp using a food mill (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 juice from peel and adhered pulp and from milled trimmings was extracted using a Wahler (Stuttgart, Germany) and a Bucher (Niederweningen, Switzerland) pack press, respectively. The juice was pasteurised at 90 °C using a pilot plant scale pasteuriser (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. Two individual batches were produced for each juice type.

2.3 Physico-chemical parameters

For the determination of total soluble solids (TSS) and pH, a digital refractometer (RX-5000; Atago, Tokyo, Japan) and pH-meter (inoLab pH 720; WTW, Weilheim, Germany), respectively, were used. Titratable acidity (TA), expressed as citric acid in g/100 mL of juice, was determined by titration with normalised 0.25 M NaOH to a pH of 8.1 using an automatic titration system (Titrino 718 STAT; Metrohm, Herisau, Switzerland). Subsequently, a formaldehyde solution adjusted to pH 8.1 was added. The formol number (FN) expressed as mL 0.1 M NaOH/100 mL juice was determined by re-titration to pH 8.1. Potassium hydrogen phthalate was used for normalisation of the titration solution. All physico-chemical parameters were determined in duplicate. The determination of total phenol content (TPC) was performed by Folin-Ciocalteu method according to Difonzo et al. (2017) with some modifications. 20 µL of juice was added to 980 µL of ddH_2O and 100 µL of Folin-Ciocalteu reagent. After 3 min, 5% Na_2CO_3 solution was added, following incubation at room temperature for 60 min. The absorbance was read at 750 nm using a spectrophotometer (Perkin Elmer, Dreieich, Germany). The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL juice.

2.4 Quantitation of vitamin C

Vitamin C, i.e., the sum of ascorbic and dehydroascorbic acid, was quantitated by HPLC-UV following a modified procedure according to Aschoff et al. (2015). Briefly, an aliquot of 0.5 mL of juice was made up to 5 mL with water containing 1.5% (*w*/*v*) meta-phosphoric acid and 20 mM tris(2-carboxyethyl)phosphine hydrochloride. The pH of the buffer was adjusted to 3.5 with aqueous 2 M K_2HPO_4 beforehand. After thorough mixing and incubation for 30 min, the samples were filtered through 0.45 µm regenerated cellulose filters (Chromafil®, Macherey-Nagel, Düren, Germany) into amber glass vials and immediately analysed by HPLC.

HPLC analyses were carried out using a Merck Hitachi LaChrom Elite HPLC system equipped with an L-2130 pump module, an L-2200 autosampler, a JetStream 2 plus column thermostat, and an L-2450

diode array detector (all from Hitachi High-Technologies, Tokyo, Japan). The HPLC system was operated with a RP-C18 column (Kinetex™, 250 × 4.6 mm, 5 µm particle size, 100 Å pore size), protected by a guard column of the same material (both from Phenomenex, Aschaffenburg, Germany). Water containing 1% (*w*/*v*) meta-phosphoric acid, adjusted to pH 2.6 with 2 M aqueous K_2HPO_4, was used as eluent, applying an isocratic elution at 25 °C. Total run time was 20 min at a flow rate of 1.0 mL/min. The injection volume was 10 µL. Ascorbic acid was detected at a wavelength of 254 nm, and quantitated by external linear calibration. The reported vitamin C concentrations equal the sum of ascorbic and dehydroascorbic acid.

2.5 HPLC-DAD-ESI-MS^n analysis

Juice samples were centrifuged for 10 min at 10.000 × *g* (MiniSpin plus, Eppendorf, Wesseling-Berzdorf, Germany) and subsequently filtered with 0.45 µm regenerated cellulose filters (Chromafil®, Macherey-Nagel, Düren, Germany). HPLC-DAD-ESI-MS^n analyses were conducted applying an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) fitted with the abovementioned RP-C18 column. Elution solvents, the gradient, and system settings were used as reported previously (Steingass et al., 2015a). Electrospray ionisation (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).

2.6 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 and mass spectrometer settings were as given above and detailed in Steingass et al. (2015a), respectively. Data evaluation was performed with Esquire Control software (Bruker).

2.7 Quantitation of amino acids, amines, furanones, and phenolic compounds by HPLC-DAD

HPLC-DAD analysis was conducted applying a Waters Acquity H-class UPLC, equipped with a quaternary solvent manager, a sample manager, and a photodiode array detector (all from Waters,

Milford, USA) applying the conditions detailed in Steingass et al. (2015a). External standards were used for quantitation using linear calibration curves. However, most of the pineapple-specific metabolites were not commercially available. Thus, structurally related compounds with a similar chromophore were used and molecular weight (MW) correction factors ($MW_{compound}/MW_{standard}$) were applied. HDMF and MDMF standards were used for the quantitation of free and glycosylated furanones. Coniferyl and *p*-coumaryl derivatives, i.e., *S*-coniferyl-L-cysteine, *S*-*p*-coumarylglutathione, *S*-coniferylglutathione, *N*-L-γ-glutamyl-*S*-coniferyl-L-cysteine were quantitated with coniferyl alcohol, the sinapyl conjugates *S*-sinapyl-L-cysteine, *S*-sinapylglutathione, *N*-L-γ-glutamyl-*S*-sinapyl-L-cysteine with sinapyl alcohol. Ferulic acid was used for (di-*E*,*E*)-*N,N′*-diferuloylspermidine quantitation, syringic acid for syringoyl hexoside, sinapic acid for sinapoyl hexoside, and caffeic acid for caffeoylisocitrate. L-Tyrosine and serotonin were quantitated using authentic reference standards. The concentrations were expressed as mg/100 mL of juice.

2.8 Statistics

The analysis of variance (ANOVA) and Tukey's test for multiple comparison were carried out using Minitab Statistical Software (Minitab Inc., State College, PA, USA) considering the pineapple tissue (*T*) and storage (*S*) as independent variables and their interaction (*T***S*); the uppercase letters indicate the statistical differences resulting from two-way ANOVA and Tukey's test above mentioned. To visualise the clustering of the samples, unsupervised principal component analysis (PCA) and hierarchical cluster analysis (HCA) were calculated with Solo software version 8.0.1 (Eigenvector Research, Wenatchee, WA, USA).

Box-plots were constructed using GraphPad software (GraphPad Software Inc., La Jolla, CA, USA). Boxes represent the lower and upper quartiles (25 and 75%), the bands inside the boxes the median (50%). Arithmetic means are indicated by cross symbols, minimum and maximum values by the whiskers, and outliers by circles.

3 Results

3.1 Pineapple juice characterisation

Table 1 summarises pH, TSS, TA, TSS/TA ratio, formol number, TPC, and vitamin C of the pineapple juice from pulp, pressed and milled peel during storage. The pH was not significantly influenced from the tissue variable (*T*). No clear trend was observed during storage. TSS were significantly higher ($p < 0.0001$) in juice from pulp (12.57 ± 0.51 to 13.07 ± 0.60 g/100 g) than from pressed and milled peel (10.74 ± 0.46 to 11.89 ± 0.35 g/100 g). No significant differences were found during storage. Both the values of TA and formol number significantly increased during storage (T14) of the peel juices, whereas they did not vary significantly in those from pulp. Differences in calculated TSS/TA ratios were found to be insignificant with the exception of juices obtained from peel after two weeks of storage (T14). Elevated TPC values of 92.06 ± 4.90 and 95.52 ± 5.11 mg GAE/100 mL were detected in milled peel juice at T7 and T14, respectively. In the pulp juice, both TPC and vitamin C significantly decreased after fourteen days (T14).

In Table S1, the physico-chemical characteristics of pineapple juice produced at a pilot plant scale were compiled. The tissue variable *T* significantly influenced all considered parameters with the exception of TA. Moreover, the juice obtained at laboratory scale at T7 showed the same trend for all the parameters. Consequently, the results from small-scale processing also apply for the juice produced at the pilot plant. However, significantly elevated concentrations of vitamin C between 57.55 ± 9.14 and 61.46 ± 9.35 mg/100 mL were found in the juice produced at pilot plant scale compared to the laboratory scale (32.57 ± 2.28 and 38.35 ± 3.59 mg/100 mL). This may be attributed to the differing thermal treatments applied.

3.2 Identification of individual compounds

Table 2 summarises the UV absorption spectra, accurate mass, and MS^n mass fragmentations of the pineapple metabolites that were assigned according to Steingass et al. (2015a) and Steingass, Glock, Lieb, & Carle (2017).

Amino acids and amines

L-Tyrosine (no. 1) had a protonated molecule $[M+H]^+$ at *m/z* 182.0813 as well as an abundant precursor ion at *m/z* 165.0547 resulting from in-source deamination ($[M+H-NH_3]^+$). CID of the protonated molecule resulted in prevailing fragment ions at *m/z* 165, 147, and 136 from the elimination of NH_3 (17 amu), H_2O (18 amu) and NH_3 well as CO (28 amu) and H_2O. CID of the $[M+H-NH_3]^+$ precursor ion at *m/z* 165 resulted in a unique product ion at *m/z* 123. Similarly, both $[M+H]^+$ and $[M+H-NH_3]^+$ at *m/z* 177.1023 and 160.0758 were detected in the MS^1 spectrum of serotonin (no. 2). CID of the $[M+H]^+$ at *m/z* 177 resulted in a single fragment ion at *m/z* 160 ($[M+H-HN_3]^+$), thus confirming the identity as an amine. L-Tryptophan (no. 6) had a deprotonated molecule $[M-H]^-$ at *m/z* 203.0820 and fragment ions at *m/z* 159 $[M-H-CO_2]^-$ and 142 ($[M-H-CO_2-NH_3]^-$) in the ESI(-)-MS^n experiment. The elimination of NH_3 was also observed in the positive ion mode. CID of the $[M+H]^+$ at *m/z* 205.0972 resulted in an abundant product ion at *m/z* 188 ($[M+H-NH_3]^+$).

Furanones

The mass spectrometric assignment of a HDMF hexoside (no. 3) and two corresponding malonyl hexosides (nos. 8 and 9) in the negative ion mode has been previously reported in detail (Steingass et al., 2017). Noteworthy, the CID of the abundant sodium adducts $[M+Na]^+$ detected in the positive ion mode at *m/z* 313.0895 (no. 3) and 399.0900 (nos. 8 and 9), respectively, resulted in a product ion at *m/z* 151 resembling $[HDMF+Na]^+$. The analogous elimination of a dehydrated hexose (162 amu) from no. 3 and dehydrated malonyl hexoses (248 amu) from nos. 8 and 9, respectively, was observed from the protonated molecules, resulting in $[HDMF+H]^+$ at *m/z* 129. The latter was detected at *m/z* 129.0548 for compound no. 4 assigned to free HDMF. CID resulted in product ions at *m/z* 111 ($[M+H-H_2O]^+$), 101 ($[M+H-CO]^+$), and 83 ($[M+H-H_2O-CO]^+$), possibly generated by water (18 amu) and carbon monoxide (28 amu) eliminations from the keto- and the hydroxyl groups of the protonated HDMF molecule. A further free furanone was assigned to the methoxy derivative MDMF (no. 7) displaying a protonated molecule $[M+H]^+$ at *m/z* 143.0704. The most abundant ion in the MS^2 spectrum was detected at *m/z* 111 ($[M+H-CH_3OH]^+$), resulting from the elimination of methanol (32 amu) from the methoxy group. Assignment of the latter two compounds as well as nos. 1, 2, and 6 was substantiated by comparing their t_R, UV, and mass spectra with those of authentic reference standards.

Phenolic compounds

The mass spectrometric assignment of *S*-sinapyl-L-cysteine (no. 12), *S*-sinapylglutathione (no. 16), and *N*-L-γ-glutamyl-*S*-sinapyl-L-cysteine (no. 19) as well as the structurally related *S*-*p*-coumaryl (no. 14) and *S*-coniferyl conjugates (nos. 11, 15, and 18) by HR-ESI-MS and MS^n experiments have been discussed in detail in our previous contributions (Steingass et al., 2015a, Steingass et al., 2017).

Compounds no. 13, and 17 were assigned to caffeoyl (no. 13) and *p*-coumaroylisocitrates (no. 17). Their assignment by HR-ESI(-)-MS and ESI(-)-MS^n experiments has been previously reported (Steingass et al., 2017). The assignment of these acyl-isocitrates was substantiated by the MS experiment in the positive ion mode. Both acyl-isocitrates displayed abundant sodium adducts $[M+Na]^+$ at *m/z* 377.0840 (no. 13) and 361.0529 (no. 17). In the MS^2 experiment, product ions at *m/z* 215 and 197 resembling the sodium adduct of isocitric ($[\text{isocitric acid}+Na]^+$) and dehydrated isocitric acid ($[\text{isocitric acid}-H_2O+Na]^+$) were detected. These product ions were generated by the elimination of caffeic (180 amu) and dehydrated caffeic acid (162 amu) from no. 13, and the corresponding *p*-coumaric acid eliminations (164 and 146 amu) from no. 17. In addition, cleavage of the ester bonds resulted in characteristic product ions resembling the sodium adducts and protonated molecules, respectively, of the dehydrated hydroxycinnamic acids. This reaction, i.e., the elimination of isocitric acid (192 amu) and dehydrated isocitric acid (174 amu) resulted in product ions at *m/z* 185 ($[\text{caffeic acid}-H_2O+Na]^+$) and 163 ($[\text{caffeic acid}-H_2O+H]^+$) in the MS^2 experiment of the caffeoyl, and *m/z* 169 ($[p\text{-coumaric acid}-H_2O+Na]^+$) and 147 ($[p\text{-coumaric acid}-H_2O+H]^+$) of the *p*-coumaroylisocitrate.

The assignment of syringoyl (no. 5) and sinapoyl hexosides (no. 10) as well as (di-*E*,*E*)-*N*,*N*′-diferuloylspermidine (no. 20) on the basis of their UV and mass spectra has been previously reported (Steingass et al., 2015a, Steingass et al., 2017). Interestingly, the phenolic glycosides no. 5 and 10 also displayed sodium adducts $[M+Na]^+$ at *m/z* 383.0949 and 409.1108, respectively, in the positive ion mode as also detected for the abovementioned phenolic esters.

3.3 Quantitation of selected compounds

Table 3 summarises the concentrations of L-tyrosine, serotonin, furanones, and phenolic compounds in pulp and peel (pressed and milled) juices. L-Tyrosine and serotonin are common pineapple juice constituents (Wen et al., 2002). Their concentrations in the juice from pulp were in accordance with those reported by others (Fedman & Lee, 1985; Wen et al., 2002). The concentration of serotonin was not significantly influenced from *T* and *S.* L-Tyrosine showed a significantly higher concentration in the milled peel juices at T7 and T14. HDMF exerting an intense aroma described as "burnt pineapple" or "fruity" and "caramel-like" has been reported for the first time in pineapples by Rodin et al. (1965). The HDMF concentrations in all types of juices significantly increased with progressing storage duration. Juice from fresh pineapple pulp contained 1.26 mg/100 mL (T7) and the concentration amounted to 2.52 mg/100 mL during storage (T14). Similarly, Brat et al. (2004) and Steingass et al. (2015b) have reported increasing HDMF concentrations with progressing maturation in the pulp of fresh pineapple fruits. Concomitantly, the concentrations of the methoxy derivative MDMF significantly increased during storage. Comparable concentrations of HDMF like those determined herein have been previously reported in the literature (Elss et al., 2005; Tokitomo, Steinhaus, Büttner, & Schieberle, 2005). Noteworthy, also the concentrations of HDMF hexoside increased during storage, whereas those of the two HDMF malonyl hexoside isomers remained constant. The highest values of the latter were observed in juice from milled peel.

In agreement with previous studies, the most abundant phenolic compounds were *S*-sinapyl-L-cysteine, *S*-sinapylglutathione, and *N*-L-γ-glutamyl-*S*-sinapyl-L-cysteine (Steingass et al., 2017; Wen, Wrolstad, & Hsu, 1999) amounting to 8.12, 4.94, and 6.97 mg/100 mL, respectively, in juice from fresh pulp. Even though no significant difference between the total concentrations of phenolic compounds determined by HPLC in pulp and peel (pressed and milled) juices was found, the individual compounds showed different trends. The variable *T* significantly ($p < 0.0001$) influenced all phenolic compounds with the exception of *p*-coumaroylisocitrate. Elevated concentrations of sinapoyl hexoside and *S*-sinapyl-L-cysteine were found in juice from pulp compared to those from pressed and milled peel. Whereas the storage duration (*S*) did not show a significant influence on sinapoyl hexoside, the concentration of *S*-sinapyl-L-cysteine increased in the pressed and milled peel juices. The opposite trend was found for *S*-

309 coniferyl-L-cysteine, caffeoylisocitrate, *N*-L-γ-glutamyl-*S*-coniferyl-L-cysteine, and (di-*E*,*E*)-*N*,*N*′-
310 diferuloylspermidine. The aforementioned compounds showed significantly higher concentrations in
311 juice from pressed and milled peel. In most instances, their concentration was higher in peel juices from
312 stored pineapples. The same trends were observed for pineapple juice produced at a pilot plant scale
313 (Table S2).
314

315 **3.4 Multivariate statistics and marker identification**

316 *Unsupervised pattern recognition by PCA and HCA*

317 In order to further explore differences among the juices, the physico-chemical parameters (Table 1) and
318 the concentrations of the pineapple juice constituents (Table 3) were subjected to HCA and PCA (Fig.
319 2). Fig. 2 a,a′ displays the PCA that was calculated on the basis of the physico-chemical parameters.
320 The first three principal components (PCs) of the model explained 78% of the variance among the
321 considered parameters with a contribution of PC1 of 44%. Two separate clusters were formed. All pulp
322 juices and those from fresh peel (pressed and milled) formed one cluster, the second comprised the peel
323 juices from stored pineapples (T7 and T14). As deduced from the location of the loadings, the parameters
324 related with juice from fresh pineapple were TSS and TSS/TA. By contrast, the remaining parameters
325 contributed to the differentiation of the peel juice from stored pineapples.
326 Moreover, HCA and PCA were calculated on the basis of the concentrations of L-tyrosine, serotonin,
327 furanones, and phenolic compounds (Table 3). All pulp samples formed one cluster, whereas a second
328 comprising pressed and milled peel juice samples was differentiated when plotting PC1 against PC2.
329 Moreover, a distribution of the samples according to the storage duration was observed (Fig. 2 b,b′).
330 Fig. 2c,c′ illustrates the PCA obtained from both physico-chemical parameters and the chemical
331 composition. The combination of both data sets resulted in a clear-cut differentiation of all pulp from
332 all peel samples as well as an arrangement of the samples according to the storage duration of the fruits.
333 Consequently, marker compounds that contribute to the differentiation of pulp and peel samples and
334 those describing the influence of storage may be deduced from this plot. The compounds with positive
335 loadings on PC2 are related with juices from pulp, those with negative loadings with pressed and milled
336 peel. In line with the results compiled in Table 3, sinapoyl hexoside (no. 10) and *S*-sinapyl-L-cysteine

337 (no. 12) were related with pulp juice showing positive loadings on PC2. *N*-L-γ-Glutamyl-*S*-coniferyl-L-
338 cysteine (no. 19) with a negative loading on PC2 contributed to the separation of the peel juice. Similarly,
339 HDMF hexoside (no. 3) and the HDMF malonyl hexosides (nos. 8 and 9) contributed to the clustering
340 described above.
341
342 *PLS-DA and PLS regression*
343
344 *Marker identification*
345 Calculating the ratios between peel- and pulp-specific compounds may permit an unambiguous
346 distinction of the juices. Selected ratios are illustrated by Fig 3. HDMF hexoside concentrations
347 increased during storage, whereas those of the two HDMF malonyl hexoside isoforms remained constant
348 (Table 3). Consequently, the calculated ratio for both pulp and peel juices showed significantly higher
349 values at T14 (Fig. 3a). Still, these ratios permitted to discriminate juices from pulp, pressed, and milled
350 peel, independently of the storage time of the pineapples.
351 The glutathione (GSH) conjugates (nos. 14, 15, and 16) had negative, the *N*-L-γ-glutamyl-L-cysteine
352 and L-cysteine derivatives (nos. 11, 12, 19, and 20) positive loadings on PC1. The ratio between the
353 aforementioned compounds was calculated that may possibly describe the effect of storage. However,
354 merely for pulp and milled peel juice a significantly higher ratio was found at T14 (Fig. 3b) due to the
355 comparatively large range of this parameter (see boxplots in Fig. 3b).
356 Caffeoylisocitrate (no. 13) is a compound related with peel juice as deduced from the loadings plot (Fig.
357 2b and c) and the concentrations compiled in Table 3. The highest concentrations were detected in milled
358 peel juice. A further acyl-isocitric acid, namely *p*-coumaroylisocitrate (no. 18) was not correlated with
359 the juice type (as shown in Table 3). Calculating the ratio between caffeoyl and *p*-coumaroylisocitrates
360 permitted the clear-cut distinction of milled peel juice (Fig. 3c).
361 In addition, the (*S*-sinapyl-L-cysteine)/*N*-L-γ-glutamyl-*S*-coniferyl-L-cysteine ratio permitted the
362 unambiguous discrimination of pulp juice from all peel-derived samples (Fig. 3d).
363

4 Discussion

Fruit juices represent an important and rapidly growing sector of the beverage industry. Similar to other high-priced food commodities, fruit juices and purees are targets for adulterations (Hong et al., 2017). Our approach for detecting the usage of juice obtained from the non-edible parts of the pineapple infructescence was based on the determination of specific markers for pulp and peel (pressed and peel) juices. Jandrić et al. (2014) used a metabolomics approach for detecting fruit juice adulterations. Moreover, Steingass et al. (2015a) have previously studied the phenolic pattern of different tissues of the pineapple infructescence and have proposed phenolic compounds as possible tools to discriminate pulp and peel. In the present study, among the physico-chemical parameters, merely TSS and TPC permitted the discrimination between pulp and peel juice (see Table 1). The metabolites detected in the juices from pulp and peel permitted to discriminate both juice categories by PCA. Moreover, this multivariate statistical tool allowed deducing specific chemical markers and ratios calculated therefrom to discriminate between the individual samples. The higher ratio of caffeoylisocitrate/*p*-coumaroylisocitrate was found to be characteristic of juice extracted from milled peel. In addition, the ratio (sinapoyl hexoside + *S*-sinapyl-L-cysteine)/*N*-L-γ-glutamyl-*S*-coniferyl-L-cysteine was significantly higher in pulp juice, thus permitted to differentiate genuine pineapple juice from minor quality juices, i.e., those from pressed peel or mill juice. Regardless of the storage time, the mean value of this ratio in juice from pulp was 11 and 6 in those from pressed and milled peel. The maximum value determined among all peel juices was 7.8. Concluding, a (sinapoyl hexoside + *S*-sinapyl-L-cysteine)/*N*-L-γ-glutamyl-*S*-coniferyl-L-cysteine ratio higher than 8 may indicate that no peel extract was added to pulp juice. Moreover, among all juice categories, the ratio HDMF hexoside/HDMF malonyl hexosides was significantly higher in the juice from pineapples processed after two weeks (T14). Consequently, this ratio may represent a suitable marker to evaluate the pineapple maturity and freshness. The applicability of the aforementioned parameters for authentication of juices produced at a large scale was successfully demonstrated (see data compiled in Table S2).

5 Conclusions

HPLC-DAD-(HR)-ESI-MS^n profiling, HPLC-DAD quantitation and subsequent PCA analysis permitted to unravel distinctive chemical markers to authenticate pineapple juices from edible pulp, pulp adhered to the peel (pressed peel), and inedible peel fractions (mill juice). Overall, the present contribution revealed distinct chemical markers to authenticate pineapple juices from peel and pulp. They may be suitable tools in the quality assurance of fruit processing companies or the official food control. Future studies may define the levels of admixed peel juice that can be evidenced using the proposed methodology, e.g., by analysing genuine pulp juice spiked with different levels of peel juices. In addition, continuative studies may further explore the applicability of the proposed indicators determined using MD2 "Extra Sweet" pineapples from Ghana, to authenticate pulp and peel juices from different genotypes, provenances or harvesting seasons.

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7 Captions

7.1 Figures

Fig. 1 HLPC-DAD chromatogram (280 nm) of pineapple (*Ananas comosus* [L.] Merr.). juice from pulp (a), pressed (b), and milled peel (c). The chemical structure of selected compounds are displayed. For peak assignment, see Table 2.

Fig. 2 Score plots of the principal component analysis (PCA) calculated on the basis of physico-chemical parameters (a), pineapple juice constituents (b) and both data sets (c). The corresponding loading plots are displayed in a′, b′, and c′. The circles in the score plots illustrate clusters from hierarchical cluster analysis (HCA). Circle, rhombus, and triangle represent juice from pulp, pressed, and milled peel, respectively. White, grey, and black colour indicate T0 (fresh pineapples), T7 (one week of storage), and T14 (two weeks of storage), respectively.

Fig. 3 Box plots illustrating the ratio of HDMF hexoside/HDMF malonyl hexosides (a), (*N*-L-γ-glutamyl-L-cysteines + L-cysteines)/GSHs (b), caffeoylisocitrate/*p*-coumaroylisocitrate (c), (sinapoyl hexoside + *S*-sinapyl-L-cysteine)/*N*-L-γ-glutamyl-*S*-coniferyl-L-cysteine (d), in juices from pulp and peel (pressed and milled). Different letters indicate significant ($p < 0.05$) differences determined by ANOVA and Tukey's test.

7.2 Tables

Table 1. Physico-chemical parameters of pineapple (*Ananas comosus* [L.] Merr.) juice from pulp and peel.

Table 2. HPLC-DAD-(HR)-ESI-MS^n data of phenolic compounds and other metabolites detected in pineapple (*Ananas comosus* [L.] Merr.) juice.

Table 3. Quantitation of phenolic compounds and other metabolites in pulp and peel juice obtained from three pineapple (*Ananas comosus* [L.] Merr.) maturity stages.

512

513 **7.3 Appendix**

514 **Table S1.** Physico-chemical parameters of pineapple (*Ananas comosus* [L.] Merr.) juice from pulp and

515 peel produced at pilot plant and laboratory scale (both T7).

516

517 **Table S2.** Quantitation of phenolic compounds and other metabolites in pineapple (*Ananas comosus* [L.]

518 Merr.) juice produced at pilot plant and laboratory scale (both T7).

Table 1.

				Pulp			Peel (pressed)			Peel (milled)		
Parameter	*T*	*S*	*T***S*	T0	T7	T14	T0	T7	T14	T0	T7	T14
pH	0.278	0.000	0.722	4.03 ± 0.02^{b}	4.12 ± 0.04^{a}	4.02 ± 0.02^{b}	4.11 ± 0.02^{a}	4.02 ± 0.01^{b}	4.10 ± 0.01^{a}	4.03 ± 0.02^{b}	4.11 ± 0.01^{a}	4.03 ± 0.01^{b}
TSS (g/100 g)	0.000	0.000	0.030	13.1 ± 0.6^{a}	12.6 ± 0.4^{a}	12.6 ± 0.5^{ab}	11.6 ± 0.7^{cd}	10.7 ± 0.5^{e}	11.0 ± 0.2^{de}	11.7 ± 0.4^{cd}	11.1 ± 0.6^{de}	11.89 ± 0.35^{bc}
TA (g/100 mL)	0.013	0.000	0.000	0.49 ± 0.02^{bc}	0.46 ± 0.07^{bc}	0.52 ± 0.03^{b}	0.43 ± 0.09^{c}	0.45 ± 0.04^{bc}	0.61 ± 0.04^{a}	0.47 ± 0.05^{bc}	0.47 ± 0.04^{bc}	0.66 ± 0.02^{a}
TSS/TA	0.000	0.000	0.012	26.5 ± 2.0^{a}	27.7 ± 3.5^{a}	24.0 ± 1.9^{a}	28.0 ± 6.6^{a}	23.9 ± 2.7^{a}	18.0 ± 1.3^{b}	25.3 ± 2.9^{a}	23.9 ± 3.0^{a}	18.03 ± 0.56^{b}
Formol number (mL 0.1 M NaOH/100 mL)	0.000	0.000	0.000	8.08 ± 0.57^{c}	8.84 ± 1.32^{c}	8.50 ± 0.55^{c}	8.26 ± 2.11^{c}	12.43 ± 0.98^{b}	12.09 ± 0.86^{b}	9.02 ± 0.94^{c}	11.46 ± 0.87^{b}	14.83 ± 1.44^{a}
TPC (mg GAE/100 mL)	0.000	0.004	0.000	78.9 ± 3.3^{d}	80.2 ± 4.7^{cd}	72.3 ± 3.3^{e}	82.8 ± 4.8^{cd}	86.2 ± 4.7^{bc}	82.3 ± 2.4^{cd}	86.2 ± 4.1^{bc}	92.1 ± 4.9^{ab}	95.5 ± 5.1^{a}
Vitamin C (mg/100 mL)	0.002	0.000	0.017	50.6 ± 6.7^{abcd}	57.6 ± 9.1^{ab}	42.0 ± 4.6^{d}	39.5 ± 6.4^{d}	59.4 ± 7.7^{ab}	42.6 ± 2.6^{cd}	48.4 ± 8.0^{bcd}	61.5 ± 9.4^{a}	53.4 ± 4.8^{abc}

T, pineapple tissue variable; *S*, storage duration of the fruits; *T***S*, interaction of the variables; T0, fresh pineapples; T7, one week of storage at room temperature; T14, two weeks of storage.
Different letters in one row indicate a significant ($p < 0.05$) difference of means determined by two-way ANOVA and Tukey's test.

Table 2.

No.	t_R (min)	λ_{max} (nm)	HR-ESI(-)-MS (*m/z*)	ESI(-)-MS^n experiment (*m/z*)	HR-ESI(+)-MS (*m/z*)	ESI(+)-MS^n experiment (*m/z*)	Proposed structure
1	6.7	274, sh281	n.d.	n.d.	182.0813 [c] (182.0812)	[182]: 165, 147, 136, 123, 119	L-Tyrosine
					165.0547 [d] (165.0546)	[165]: 147, 123	
2	8.1	277, sh298	n.d.	n.d.	177.1023 [c] (177.1022)	[177]: 160	Serotonin
					160.0758 [d] (160.0756)	[160]: 95	
3	12.9	276	289.0930 [a] (289.0929)	[289]: 161, 127, 113, 101	313.0895 [c] (313.0894)	[313]: 184, 169, 151	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone hexoside
					291.1075 [c] (291.1074)	[291]: 129	
4	13.2	287	n.d.	n.d.	129.0548 [c] (129.0546)	[129]: 111, 101, 87, 83	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone
5	17.8	279	359.0988 [a] (359.0984)	[359]: 197	383.0949 [e] (383.0949)	[383]: 278, 221, 185	Syringoyl hexoside
6	17.8	sh271, 279, 288	203.0820 [a] (203.0826)	[203]: 159, 142, 116	205.0972 [c] (205.0972)	[205]: 188, 146	L-Tryptophan
					188.0706 [d] (188.0706)	[188]: 146, 119	
7	21.7	279	n. d.	n. d.	143.0704 [c] (143.0703)	[143]: 129, 111, 83	4-Methoxy-2,5-dimethyl-3(2*H*)-furanone
8	21.9	277	331.1037 [b] (331.1035)	[331]: 289, 271, 203, 193, 169, 161, 127, 113, 101	399.0900 [e] (399.0898)	[399]: 355, 151	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone malonyl hexoside (1)
					377.1080 [c] (377.1078)	[377]: 129	
9	22.4	277	331.1037 [b] (331.1035)	[331]: 289, 271, 203, 193, 169, 161, 127, 113, 101	399.0900 [e] (399.0898)	[399]: 355, 151	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone malonyl hexoside (2)
					377.1080 [c] (377.1078)	[377]: 129	
10	24.4	331	385.1145 [a] (385.1140)	[385]: 223, 205	409.1108 [e] (409.1105)	[409]: 247, 185	Sinapoyl hexoside
11	25.8	268, sh303	n. d.	n. d.	284.0947 [c] (284.0951)	[284]: 163, 131, 103	*S*-Coniferyl-L-cysteine
12	26.3	282	n. d.	n. d.	314.1052 [c] (314.1057)	[314]: 193, 161, 133	*S*-Sinapyl-L-cysteine
13	28.1	299, 328	353.0516 [a] (353.0514)	[353]: 191, 173, 155, 111	377.0840 [e] (377.0479)	[377]: 215, 197, 185, 163	Caffeoylisocitrate
14	30.3	266, sh300	438.1347 [a] (438.1340)	[438]: 306, 288, 254	440.1482 [c] (440.1486)	[440]: 308, 179, 162	*S*-*p*-Coumarylglutathione
15	31.8	269, sh303	468.1451 [a] (468.1446)	[468]: 306, 288, 254	470.1588 [e] (470.1592)	[470]: 308, 179, 162	*S*-Coniferylglutathione
16	32.2	279	498.1558 [a] (498.1552)	[498]: 306, 288, 254	500.1694 [c] (500.1697)	[500]: 308, 179, 162	*S*-Sinapylglutathione
17	33.8	315	337.0568 [a] (337.0565)	[337]: 173, 155, 111	361.0529 [e] (361.0530)	[361]: 215, 197, 169, 147	*p*-Coumaroylisocitrate
18	33.9	269, sh303	411.1238 [a] (411.1231)	[411]: 281, 249, 128	413.1371 [c] (413.1377)	[413]: 251, 163, 131	*N*-L-γ-Glutamyl-*S*-coniferyl-L-cysteine
19	34.2	281	441.1339 [a] (441.1337)	[441]: 249, 153, 128	443.1478 [c] (443.1483)	[443]: 251, 193, 161	*N*-L-γ-Glutamyl-*S*-sinapyl-L-cysteine
20	37.8	sh294, 319	496.2458 [a] (496.2453)	[496]: 346	498.2594 [c] (498.2599)	[498]: 481, 322, 234, 177	(di-*E*,*E*)-*N*,*N*′-Diferuloylspermidine

t_R: retention time. λ_{max}: UV maxima (sh: shoulder). n. d.: not detected. Calculated exact masses are given in parenthesis.

[a] *m/z* of $[M-H]^-$

[b] In-source decarboxylation ($[M-H-CO_2]^-$)

[c] *m/z* of $[M+H]^+$

[d] In-source deamination $[M+H-NH_3]^+$

[e] Sodium adduct $[M+Na]^+$

Table 3.

				Pulp			Peel (pressed)			Peel (milled)		
Compounds	*T*	*S*	*T***S*	T0	T7	T14	T0	T7	T14	T0	T7	T14
Amino acids and amines												
L-Tyrosine	0.000	0.000	0.119	3.97 ± 0.42^{c}	4.54 ± 1.21^{bc}	4.00 ± 0.55^{c}	4.49 ± 0.60^{bc}	5.32 ± 1.39^{ab}	5.46 ± 0.64^{ab}	4.98 ± 0.56^{bc}	6.52 ± 0.84^{a}	6.54 ± 1.15^{a}
Serotonin	0.083	0.115	0.451	1.56 ± 0.22^{a}	1.90 ± 0.12^{a}	1.89 ± 0.12^{a}	1.75 ± 0.35^{a}	1.96 ± 0.48^{a}	2.09 ± 0.16^{a}	2.55 ± 0.35^{a}	2.94 ± 0.31^{a}	2.91 ± 0.36^{a}
Total concentration	0.000	0.001	0.868	5.53 ± 0.50^{b}	6.44 ± 1.16^{b}	7.29 ± 4.16^{ab}	6.24 ± 0.76^{b}	7.28 ± 1.73^{ab}	7.54 ± 0.71^{ab}	7.53 ± 0.87^{ab}	9.46 ± 0.83^{a}	9.45 ± 1.29^{a}
Furanones												
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone hexoside	0.000	0.000	0.044	5.86 ± 0.45^{c}	6.87 ± 0.59^{b}	7.89 ± 0.40^{a}	4.56 ± 0.54^{d}	4.95 ± 0.62^{d}	6.33 ± 0.28^{bc}	4.39 ± 0.55^{d}	4.91 ± 0.54^{d}	6.84 ± 0.48^{b}
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	0.000	0.000	0.514	1.26 ± 0.14^{c}	1.98 ± 0.09^{b}	2.52 ± 0.11^{a}	0.86 ± 0.13^{d}	1.43 ± 0.16^{c}	2.09 ± 0.18^{b}	0.81 ± 0.35^{d}	1.49 ± 0.17^{c}	2.20 ± 0.29^{b}
4-Methoxy-2,5-dimethyl-3(2*H*)-furanone	0.000	0.000	0.168	0.28 ± 0.04^{d}	0.38 ± 0.05^{cd}	0.51 ± 0.08^{b}	0.33 ± 0.05^{d}	0.46 ± 0.12^{bc}	0.64 ± 0.03^{a}	0.32 ± 0.09^{d}	0.46 ± 0.09^{bc}	0.68 ± 0.12^{a}
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone malonyl hexoside (1)	0.000	0.885	0.000	1.45 ± 0.11^{b}	1.48 ± 0.10^{b}	1.26 ± 0.11^{b}	1.44 ± 0.23^{b}	1.29 ± 0.22^{b}	1.35 ± 0.11^{b}	1.76 ± 0.16^{a}	1.85 ± 0.20^{a}	1.99 ± 0.08^{a}
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone malonyl hexoside (2)	0.000	0.038	0.114	1.59 ± 0.30^{b}	1.93 ± 0.27^{b}	1.86 ± 0.11^{b}	1.86 ± 0.45^{b}	1.82 ± 0.16^{b}	1.85 ± 0.32^{b}	2.36 ± 0.25^{a}	2.44 ± 0.30^{a}	2.69 ± 0.17^{a}
Total concentration	0.000	0.000	0.019	10.44 ± 0.82^{ef}	12.65 ± 0.91^{bc}	14.03 ± 0.58^{ab}	9.05 ± 1.21^{f}	9.95 ± 1.09^{ef}	12.25 ± 0.49^{cd}	9.63 ± 1.31^{f}	11.15 ± 1.15^{de}	14.38 ± 0.77^{a}
Phenolic compounds												
Syringoyl hexoside	0.000	0.349	0.001	2.76 ± 0.50^{a}	2.53 ± 0.44^{ab}	2.38 ± 0.23^{ab}	2.13 ± 0.29^{b}	2.14 ± 0.37^{b}	2.32 ± 0.23^{ab}	2.15 ± 0.11^{b}	2.55 ± 0.29^{ab}	2.71 ± 0.20^{a}
Sinapoyl hexoside	0.000	0.500	0.460	5.68 ± 0.51^{ab}	5.90 ± 0.65^{a}	5.97 ± 0.52^{a}	4.71 ± 0.86^{c}	4.46 ± 0.77^{c}	4.69 ± 0.42^{c}	4.53 ± 0.71^{c}	5.07 ± 0.63^{abc}	4.81 ± 0.59^{bc}
S-Coniferyl-L-cysteine	0.000	0.000	0.039	0.70 ± 0.11^{d}	0.85 ± 0.14^{cd}	0.89 ± 0.07^{cd}	0.82 ± 0.11^{cd}	0.99 ± 0.15^{bc}	1.15 ± 0.21^{ab}	0.83 ± 0.48^{cd}	1.22 ± 0.23^{a}	1.30 ± 0.17^{a}
S-Sinapyl-L-cysteine	0.000	0.000	0.228	8.12 ± 1.56^{ab}	8.46 ± 1.12^{a}	8.73 ± 0.46^{a}	5.77 ± 0.87^{cd}	6.62 ± 1.19^{bcd}	7.45 ± 0.82^{ab}	5.13 ± 0.57^{d}	6.74 ± 1.42^{bc}	7.23 ± 1.01^{abc}
Caffeoylisocitrate	0.000	0.000	0.000	1.22 ± 0.28^{f}	1.43 ± 0.23^{f}	2.32 ± 0.17^{de}	1.63 ± 0.47^{ef}	1.94 ± 0.37^{def}	2.55 ± 0.45^{d}	3.59 ± 0.82^{c}	4.98 ± 0.57^{b}	6.38 ± 0.82^{a}
S-*p*-Coumarylglutathione	0.000	0.007	0.001	0.24 ± 0.05^{a}	0.24 ± 0.07^{a}	0.18 ± 0.02^{bcd}	0.15 ± 0.03^{cde}	0.21 ± 0.06^{ab}	0.21 ± 0.03^{abc}	0.13 ± 0.01^{de}	0.14 ± 0.03^{de}	0.10 ± 0.01^{e}
S-Coniferylglutathione	0.000	0.000	0.782	0.79 ± 0.13^{ab}	0.66 ± 0.20^{bc}	0.54 ± 0.06^{c}	1.02 ± 0.16^{a}	0.84 ± 0.30^{ab}	0.78 ± 0.04^{abc}	0.98 ± 0.09^{a}	0.88 ± 0.18^{ab}	0.67 ± 0.11^{bc}
S-Sinapylglutathione	0.000	0.000	0.328	4.94 ± 0.60^{ab}	4.15 ± 1.21^{bc}	3.28 ± 0.35^{cd}	5.44 ± 0.76^{a}	4.68 ± 1.26^{ab}	4.44 ± 0.31^{abc}	4.90 ± 0.67^{ab}	4.18 ± 0.67^{bc}	2.95 ± 0.69^{d}
p-Coumaroylisocitrate	0.095	0.000	0.162	2.90 ± 0.35^{d}	3.28 ± 0.22^{bcd}	4.69 ± 0.92^{a}	2.32 ± 0.15^{d}	3.14 ± 0.32^{cd}	4.26 ± 0.91^{ab}	3.00 ± 0.57^{cd}	3.05 ± 1.11^{cd}	3.97 ± 0.65^{abc}
N-L-γ-Glutamyl-*S*-coniferyl-L-cysteine	0.000	0.001	0.000	1.33 ± 0.16^{de}	1.27 ± 0.14^{de}	1.20 ± 0.12^{e}	1.74 ± 0.19^{bc}	1.85 ± 0.29^{abc}	2.01 ± 0.25^{ab}	1.59 ± 0.22^{cd}	2.05 ± 0.30^{ab}	2.11 ± 0.29^{a}
N-L-γ-Glutamyl-*S*-sinapyl-L-cysteine	0.000	0.017	0.013	6.97 ± 0.78^{abc}	6.79 ± 0.61^{abc}	6.09 ± 0.24^{bc}	7.07 ± 0.82^{abc}	8.07 ± 1.55^{a}	8.26 ± 0.73^{a}	5.90 ± 0.66^{c}	7.59 ± 1.32^{ab}	7.04 ± 1.79^{abc}
(di-*E*,*E*)-*N*,*N*′-Diferuloylspermidine	0.000	0.019	0.000	0.23 ± 0.03^{e}	0.18 ± 0.09^{e}	0.18 ± 0.03^{e}	0.32 ± 0.04^{bcd}	0.30 ± 0.05^{cd}	0.28 ± 0.01^{d}	0.34 ± 0.03^{bc}	0.38 ± 0.02^{a}	0.35 ± 0.04^{ab}
Total concentration	0.141	0.000	0.013	35.87 ± 2.90^{ab}	35.75 ± 2.47^{ab}	36.44 ± 1.90^{ab}	33.10 ± 2.90^{b}	35.23 ± 3.24^{ab}	38.39 ± 2.18^{a}	33.07 ± 3.40^{b}	38.83 ± 3.75^{a}	39.63 ± 3.90^{a}

The results are expressed as mg/100 mL.
T, pineapple tissue variable, *S*, storage duration of the fruits; *T***S*, interaction of the variables; T0, fresh pineapples; T7, one week of storage at room temperature; T14, two weeks of storage.
Different letters in one row indicate a significant ($p < 0.05$) difference of means determined by two-way ANOVA and Tukey's test.

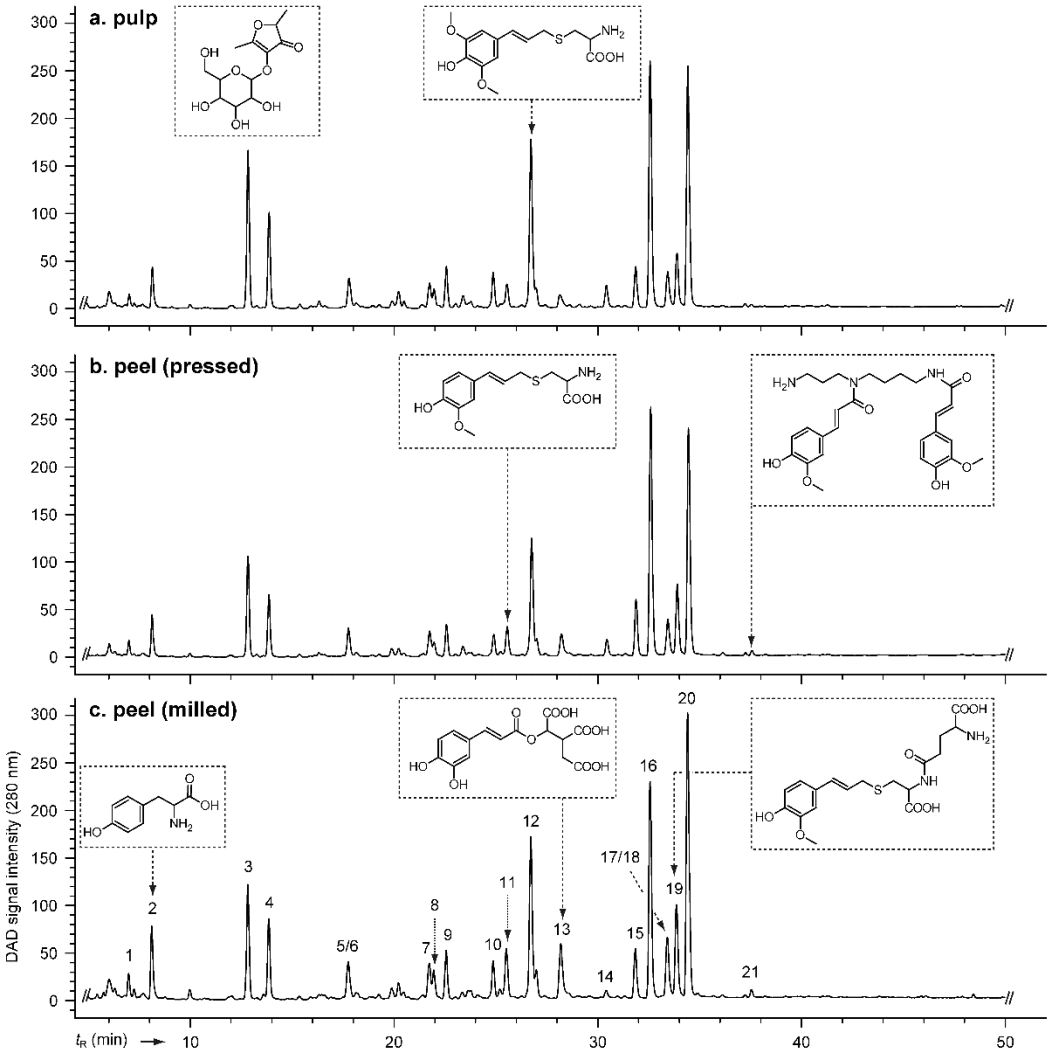

a. pulp
b. peel (pressed)
c. peel (milled)
DAD signal intensity (280 nm)
t_R (min)
1
2
3
4
5/6
7
8
9
10
11
12
13
14
15
16
17/18
19
20
21

Figure 1

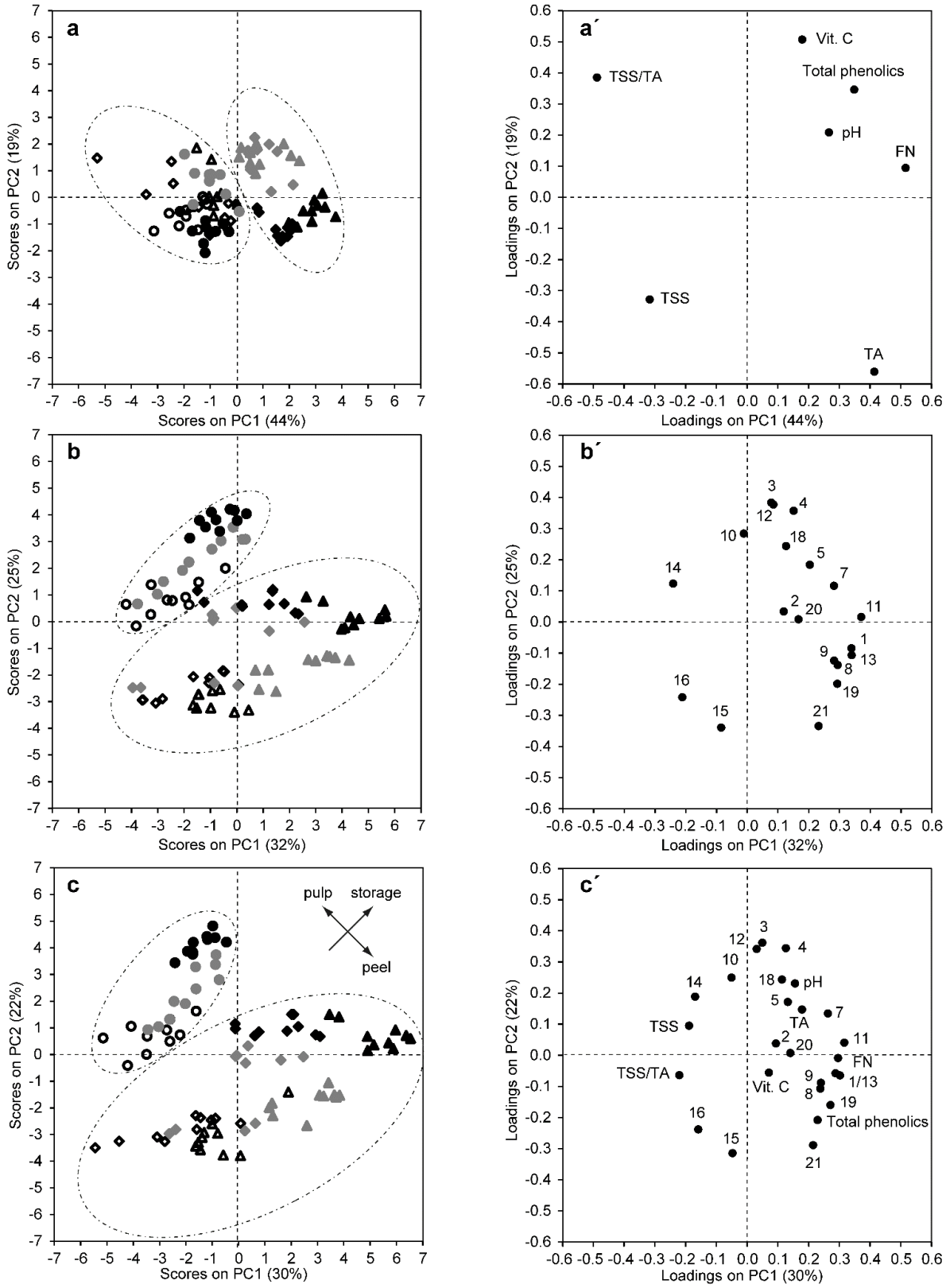
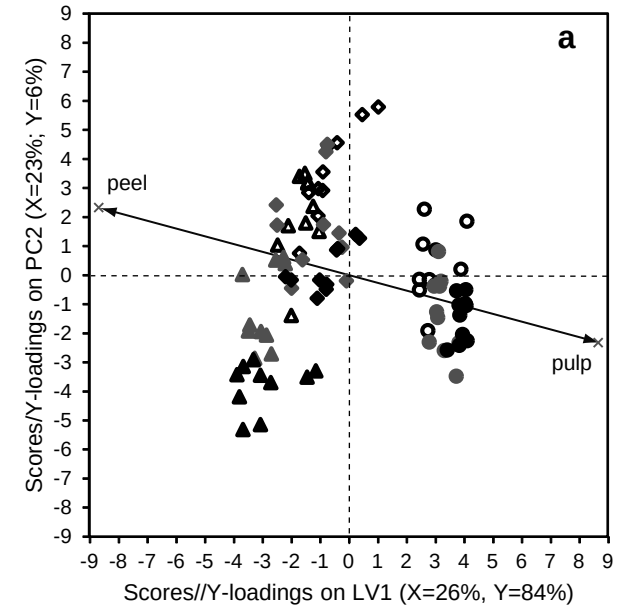
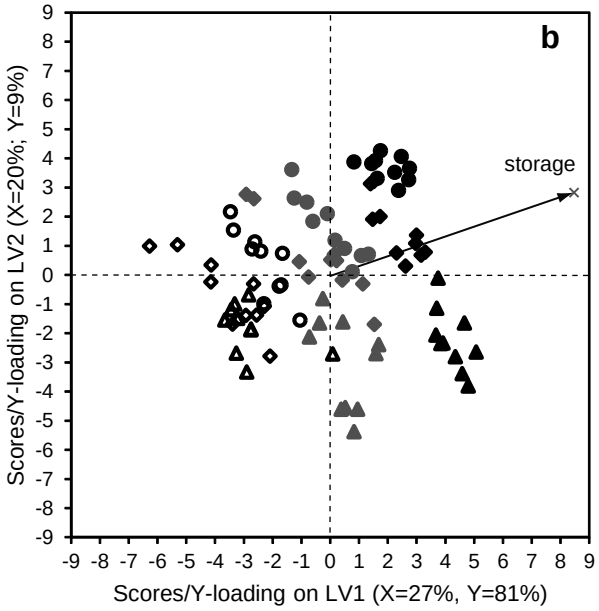
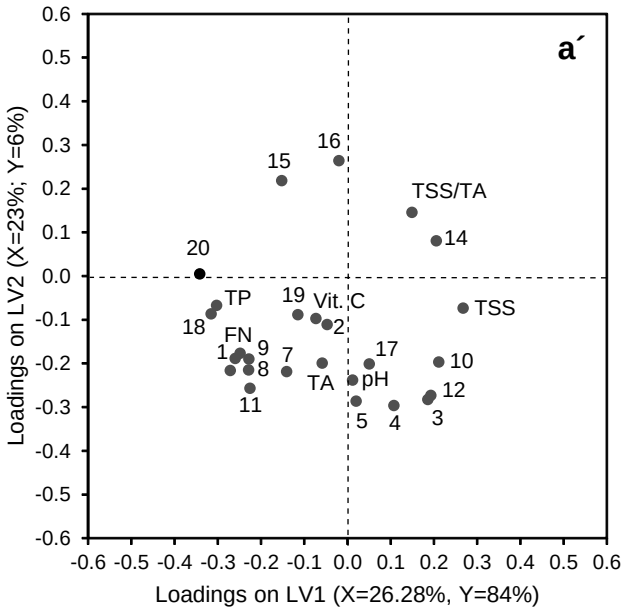
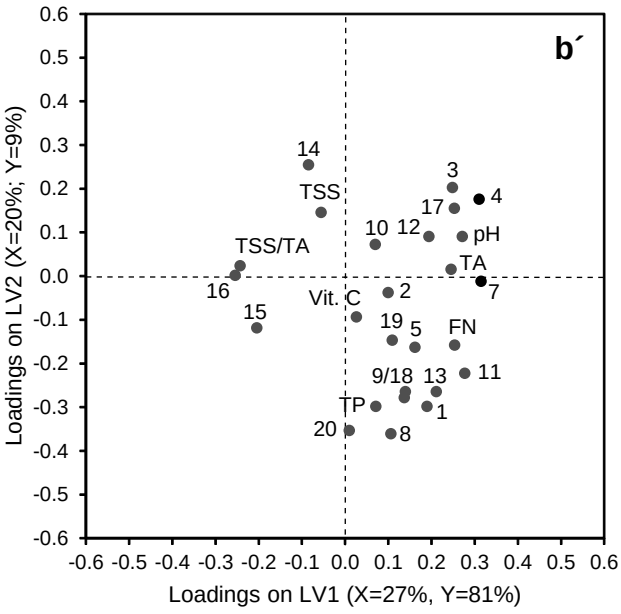

a
Scores on PC2 (19%)
Scores on PC1 (44%)
a´
Vit. C
TSS/TA
Total phenolics
pH
FN
TSS
TA
Loadings on PC2 (19%)
Loadings on PC1 (44%)
b
Scores on PC2 (25%)
Scores on PC1 (32%)
b´
Loadings on PC2 (25%)
Loadings on PC1 (32%)
c
pulp
storage
peel
Scores on PC2 (22%)
Scores on PC1 (30%)
c´
Loadings on PC2 (22%)
Loadings on PC1 (30%)

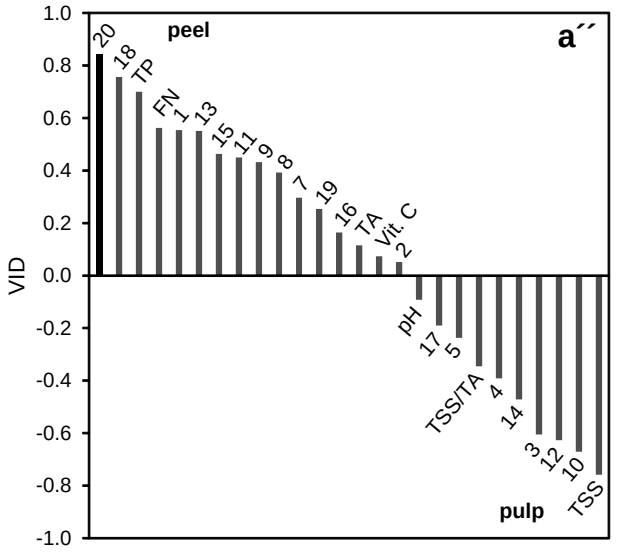
Figure 2

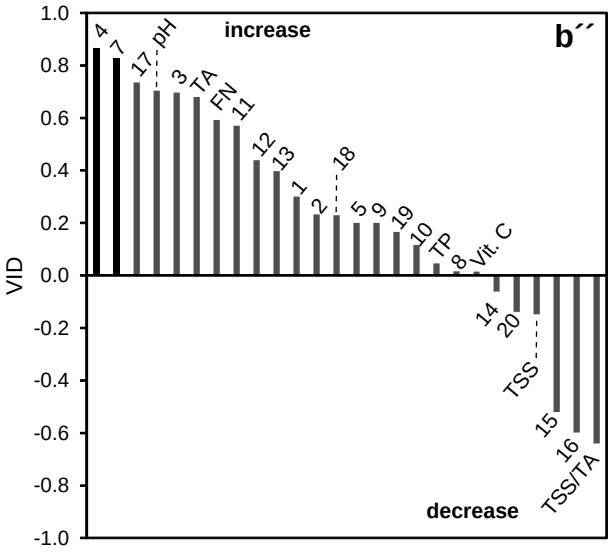

a
peel
pulp
Scores/Y-loadings on PC2 (X=23%; Y=6%)
Scores//Y-loadings on LV1 (X=26%, Y=84%)

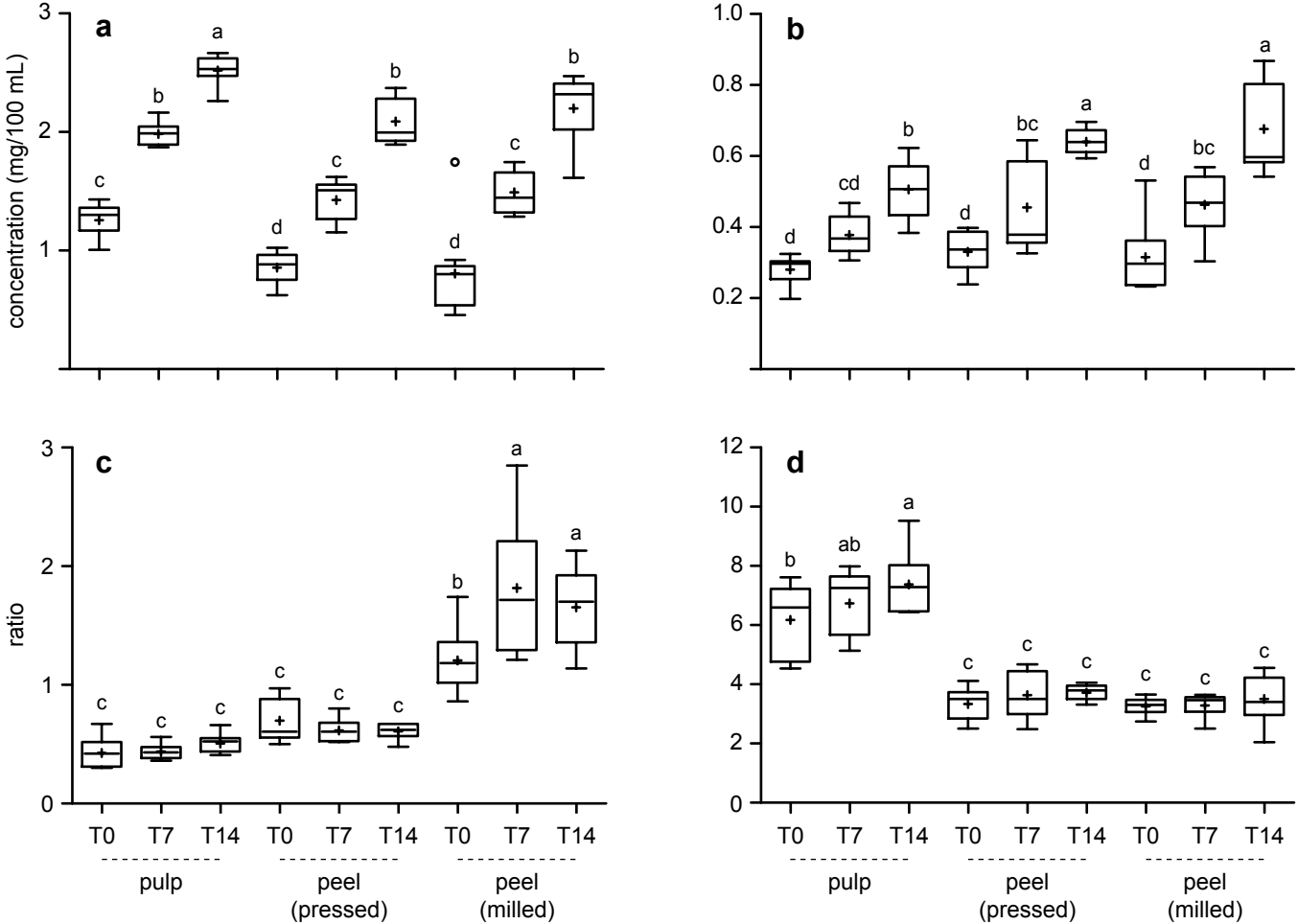

b
storage
Scores/Y-loading on LV2 (X=20%; Y=9%)
Scores/Y-loading on LV1 (X=27%, Y=81%)


a´
TSS/TA
TSS
TP
FN
TA
pH
Vit. C
Loadings on LV2 (X=23%; Y=6%)
Loadings on LV1 (X=26.28%, Y=84%)


b´
TSS
TSS/TA
pH
TA
Vit. C
FN
TP
Loadings on LV2 (X=20%; Y=9%)
Loadings on LV1 (X=27%, Y=81%)


a´´
peel
pulp
VID


b´´
increase
decrease
VID


a
concentration (mg/100 mL)
3
2
1
c
b
a
d
c
b
d
c
b
b
c
d
cd
b
d
bc
a
d
bc
a
1.0
0.8
0.6
0.4
0.2
c
ratio
3
2
1
0
c
c
c
c
c
c
b
a
a
d
12
10
8
6
4
2
0
b
ab
a
c
c
c
c
c
c
T0
T7
T14
pulp
peel
(pressed)
peel
(milled)

Supplementary data

Authentication of pineapple (*Ananas comosus* [L.] Merr.) juice from pulp and peel by HPLC-DAD-(HR)-ESI-MS^n analysis

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Supplementary data

Table S1.

		Pilot plant (T7)			Laboratory scale (T7)		
Parameter	*T*	Pulp	Peel (pressed)	Peel (milled)	Pulp	Peel (pressed)	Peel (milled)
pH	0.009	4.11 ± 0.03^{ab}	4.08 ± 0.04^{b}	4.08 ± 0.03^{b}	4.12 ± 0.04^{ab}	4.02 ± 0.01^{b}	4.11 ± 0.01^{ab}
TSS (g/100 g)	0.000	12.8 ± 0.4^{a}	9.4 ± 0.3^{d}	10.3 ± 0.3^{c}	12.6 ± 0.4^{a}	10.7 ± 0.5^{bc}	11.1 ± 0.6^{b}
TA (g/100 mL)	0.960	0.48 ± 0.04^{a}	0.46 ± 0.04^{a}	0.47 ± 0.03^{a}	0.46 ± 0.07^{a}	0.45 ± 0.04^{a}	0.47 ± 0.04^{a}
TSS/TA	0.000	26.9 ± 2.6^{ab}	20.5 ± 2.0^{c}	22.3 ± 1.7^{bc}	27.7 ± 3.5^{a}	23.9 ± 2.7^{abc}	23.9 ± 3.0^{abc}
Formol number (mL 0.1 M NaOH/100 mL)	0.000	9.26 ± 0.76^{b}	12.03 ± 0.80^{a}	12.30 ± 0.68^{a}	8.84 ± 1.32^{b}	12.43 ± 0.98^{a}	11.46 ± 0.87^{a}
TPC (mg GAE/100 mL)	0.000	84.3 ± 4.6^{cd}	89.9 ± 4.8^{bc}	100.6 ± 3.3^{a}	80.2 ± 4.7^{d}	86.2 ± 4.7^{bc}	92.1 ± 4.9^{b}
Vitamin C (mg/100 mL)	0.000	38.4 ± 3.6^{b}	32.6 ± 2.3^{b}	35.4 ± 1.6^{b}	57.6 ± 9.1^{a}	59.4 ± 7.7^{a}	61.5 ± 9.4^{a}

T, pineapple tissue variable; T7, one week of storage
Different letters in row indicate a significant ($p < 0.05$) difference of means determined by ANOVA and Tukey's test.

Table S2.

		Pilot plant (T7)			Laboratory scale (T7)		
Compounds	*T*	Pulp	Peel (pressed)	Peel (milled)	Pulp	Peel (pressed)	Peel (milled)
Amino acids and amines							
L-Tyrosine	0.000	3.84 ± 0.14^{b}	5.21 ± 0.33^{ab}	4.96 ± 0.45^{b}	4.54 ± 1.21^{b}	5.32 ± 1.39^{ab}	6.52 ± 0.84^{a}
Serotonin	0.000	1.60 ± 0.09^{c}	2.09 ± 0.20^{bc}	2.60 ± 0.02^{ab}	1.90 ± 0.12^{c}	1.96 ± 0.48^{c}	2.94 ± 0.31^{a}
Total concentration	0.000	5.44 ± 0.17^{c}	7.30 ± 0.38^{b}	7.55 ± 0.46^{b}	6.44 ± 1.16^{c}	7.28 ± 1.73^{b}	9.46 ± 0.83^{a}
Furanones							
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone hexoside	0.000	7.30 ± 0.10^{a}	5.81 ± 0.26^{b}	5.95 ± 0.17^{b}	6.87 ± 0.59^{a}	4.95 ± 0.62^{c}	4.91 ± 0.54^{c}
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	0.000	2.12 ± 0.03^{a}	1.73 ± 0.10^{b}	1.74 ± 0.04^{b}	1.98 ± 0.09^{a}	1.43 ± 0.16^{c}	1.49 ± 0.17^{c}
4-Methoxy-2,5-dimethyl-3(2*H*)-furanone	0.000	0.42 ± 0.04^{ab}	0.55 ± 0.03^{a}	0.56 ± 0.03^{a}	0.38 ± 0.05^{b}	0.46 ± 0.12^{ab}	0.46 ± 0.09^{ab}
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone malonyl hexoside (1)	0.000	1.65 ± 0.04^{bc}	1.78 ± 0.10^{b}	2.31 ± 0.13^{a}	1.48 ± 0.10^{cd}	1.29 ± 0.22^{d}	1.85 ± 0.20^{b}
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone malonyl hexoside (2)	0.000	2.09 ± 0.36^{cd}	2.57 ± 0.15^{b}	3.12 ± 0.19^{a}	1.93 ± 0.27^{d}	1.82 ± 0.16^{d}	2.44 ± 0.30^{bc}
Total concentration	0.000	13.58 ± 0.38^{a}	12.44 ± 0.62^{ab}	13.69 ± 0.55^{a}	12.65 ± 0.91^{a}	9.95 ± 1.09^{c}	11.15 ± 1.15^{bc}
Phenolic compounds							
Syringoyl hexoside	0.066	2.54 ± 0.07^{a}	2.47 ± 0.17^{a}	2.39 ± 0.06^{a}	2.53 ± 0.44^{a}	2.14 ± 0.37^{a}	2.55 ± 0.29^{a}
Sinapoyl hexoside	0.000	6.16 ± 0.12^{a}	4.44 ± 0.25^{bc}	4.04 ± 0.04^{c}	5.90 ± 0.65^{a}	4.46 ± 0.77^{bc}	5.07 ± 0.63^{b}
S-Coniferyl-L-cysteine	0.000	0.89 ± 0.07^{b}	1.05 ± 0.06^{ab}	1.01 ± 0.03^{ab}	0.85 ± 0.14^{b}	0.99 ± 0.15^{b}	1.22 ± 0.23^{a}
S-Sinapyl-L-cysteine	0.000	8.53 ± 0.42^{a}	6.49 ± 0.21^{b}	5.60 ± 0.11^{b}	8.46 ± 1.12^{a}	6.62 ± 1.19^{b}	6.74 ± 1.42^{b}
Caffeoylisocitrate	0.000	1.92 ± 0.08^{d}	3.96 ± 0.36^{c}	7.67 ± 0.57^{a}	1.43 ± 0.23^{d}	1.94 ± 0.37^{d}	4.98 ± 0.57^{b}
S-*p*-Coumarylglutathione	0.000	0.25 ± 0.01^{a}	0.12 ± 0.02^{b}	0.13 ± 0.00^{b}	0.24 ± 0.07^{a}	0.21 ± 0.06^{a}	0.14 ± 0.03^{b}
S-Coniferylglutathione	0.025	0.77 ± 0.01^{ab}	1.01 ± 0.06^{a}	0.92 ± 0.04^{ab}	0.66 ± 0.20^{b}	0.84 ± 0.30^{ab}	0.88 ± 0.18^{ab}
S-Sinapylglutathione	0.587	4.52 ± 0.11^{a}	4.84 ± 0.35^{a}	4.32 ± 0.05^{a}	4.15 ± 1.21^{a}	4.68 ± 1.26^{a}	4.18 ± 0.67^{a}
p-Coumaroylisocitrate	0.000	4.32 ± 0.24^{a}	3.55 ± 0.18^{ab}	2.31 ± 0.52^{c}	3.28 ± 0.22^{b}	3.14 ± 0.32^{bc}	3.05 ± 1.11^{bc}
N-L-γ-Glutamyl-*S*-coniferyl-L-cysteine	0.000	1.40 ± 0.02^{b}	2.16 ± 0.13^{a}	1.89 ± 0.09^{a}	1.27 ± 0.14^{b}	1.85 ± 0.29^{a}	2.05 ± 0.30^{a}
N-L-γ-Glutamyl-*S*-sinapyl-L-cysteine	0.025	6.61 ± 0.15^{a}	7.56 ± 0.64^{a}	6.49 ± 0.08^{a}	6.79 ± 0.61^{a}	8.07 ± 1.55^{a}	7.59 ± 1.32^{a}
(di-*E*,*E*)-*N*,*N*′-Diferuloylspermidine	0.000	0.18 ± 0.01^{d}	0.23 ± 0.04^{c}	0.22 ± 0.00^{cd}	0.18 ± 0.09^{d}	0.30 ± 0.05^{b}	0.38 ± 0.02^{a}
Total concentration	0.057	38.10 ± 1.10^{a}	38.02 ± 1.82^{a}	36.99 ± 0.34^{a}	35.75 ± 2.47^{a}	35.23 ± 3.24^{a}	38.83 ± 3.75^{a}
Ratio							
HDMF hexoside/HDMF malonyl hexosides	0.000	1.97 ± 0.23^{a}	1.34 ± 0.02^{b}	1.10 ± 0.04^{c}	2.01 ± 0.08^{a}	1.59 ± 0.11^{b}	1.14 ± 0.05^{c}
(*N*-L-γ-glutamyl-L-cysteines + L-cysteines)/GSHs	0.327	3.15 ± 0.04^{a}	2.89 ± 0.10^{a}	2.79 ± 0.02^{a}	3.79 ± 1.24^{a}	3.38 ± 1.24^{a}	3.50 ± 0.90^{a}
Caffeoylisocitrate/*p*-coumaroylisocitrate	0.000	0.45 ± 0.04^{c}	1.12 ± 0.11^{bc}	3.56 ± 1.05^{a}	0.44 ± 0.07^{c}	0.62 ± 0.10^{c}	1.82 ± 0.58^{b}
Sinapoyl hexoside + *S*-sinapyl- L-cysteine/*N*- L-γ-glutamyl-*S*-coniferyl- L-cysteine	0.000	10.51 ± 0.33^{a}	5.06 ± 0.11^{b}	5.11 ± 0.32^{b}	11.42 ± 1.62^{a}	6.07 ± 1.26^{b}	5.78 ± 0.56^{b}

The results are expressed as mg/100 mL. *T*, pineapple tissue variable; T7, one week of storage at room temperature.
Different letters in one row indicate a significant ($p < 0.05$) difference of means determined by ANOVA and Tukey's test.