Food Chemistry 324 (2020) 126874

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chemical composition of thermally processed coconut water evaluated by GC–MS, UPLC-HRMS, and NMR

Aline G. Cunha[a], Elenilson G. Alves Filho[b], Lorena Mara A. Silva[c], Paulo Riceli V. Ribeiro[c], Tigressa Helena S. Rodrigues[d], Edy S. de Brito[c], Maria Raquel A. de Miranda[a,*]

[a] Universidade Federal do Ceará, Dept. of Biochemistry and Molecular Biology, Campus Pici, Av. Mister Hull 2297 Bl. 907, Fortaleza-CE, CEP 60440-554, Brazil
[b] Dept. of Food Engineering, Universidade Federal do Ceará, Campus do Pici, Bloco 858, Fortaleza-CE 60440-900, Brazil
[c] Embrapa Agroindústria Tropical, Rua Dra. Sara Mesquita 2270, Fortaleza-CE, CEP 60511-110, Brazil
[d] Universidade Estadual do Vale do Acaraú-UVA, Sobral-CE, Brazil

ARTICLE INFO

Keywords:
Cocos nucifera
Sterilization
Multivariate analysis
Metabolites

ABSTRACT

Thermally-processed coconut water often develop a commercially-undesirable pink color, thus, NMR, UPLC-HRMS, GC–MS analyses combined with chemometrics approach were applied to evaluate chemical variations in comparison to tender water (control) that could explain such color change. Chemometrics on negative ionization mode dataset showed trimeric and A-type dimeric procyanidins, and caffeoylshikimic acid as main identified secondary metabolites induced by processing, while, control water presented mainly cytokinin *trans*-zeatin riboside, procyanidin dimer, caffeoylshikimic acid and trihydroxy-octadecenoic acid. Processing increased long-chain saturated palmitic and stearic fatty acids contents, meanwhile NMR analysis showed a decline in primary metabolites content as sugars fructose and glucose, and short-chain organic acids. Among the results observed for thermally processed coconut water, the increase in oligomeric procyanidins as A-type dimer and trimer may be associated with pink color development as these are precursors of anthocyanin pigment and/or by enhancing color stability of anthocyanin solutions.

1. Introduction

Water collected from green coconut is a natural drink widely consumed due to its health-associated nutrients as proteins, lipids, minerals, carbohydrates, and organic acids (Debmandal & Mandal, 2011; Jirapong, Wongs-Aree, Noichinda, Uthairatanakij, & Kanlayanarat, 2015; Rolle, 2007; Santoso, Kubo, Ota, Tadokoro, & Maekawa, 1996). However, studies on coconut water chemical composition have been limited to constituents with nutritional relevance, although a greater diversity of compounds contribute to its special biological properties.

Coconut water components have been analyzed by different techniques for various purposes as changes in quality variables under different sterilization processes, by nuclear magnetic resonance (NMR) spectroscopy with chemometrics (Porto et al., 2020; Sucupira et al., 2017); by gas chromatography (GC) to characterize volatile (aroma) profile (Prades, Assa, Dornier, Pain, & Boulanger, 2012) and high performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) to analyze different classes of phytohormones (Ma et al., 2008). Chen, Zhang, Chen, Zhongand, and Chen (2018) applied a metabolomic technique based on UPLC-MS/MS and multivariate statistics to reveal changes of metabolites during postharvest storage of matured coconut water involved with deterioration and metabolic regulation. The authors reported that water should be stored for no longer than 4 months under room conditions based on 12 biomarkers identified, e.g. taurine, pantothenic acid and malic acid.

Water is the liquid endosperm of immature coconuts and within a day after collection from the nut and exposure to air, it undergoes chemical reactions and may develop a pink color (Prades, Dornier, Diop, & Pain, 2012). Coconut water discoloration has been associated with microbial or enzymatic browning activities (Murasaki-Aliberti, Silva, Gut, & Tadini, 2009), however, this was discarded as results showed that boiling did not prevent pink color development (Damar, Balaban, & Sims, 2009). Besides this, thermal processing is the most common preservation method in the food industry due to its effectiveness in microbiological and enzymatic control, thus enabling long-term commercialization (Awuah, Ramaswamy, & Economides, 2007; de Aguiar, Yamashita, & Gut, 2012). However, thermal processing also induces discoloration reactions with pink color development leading to decline of commercial quality and acceptance, during storage of coconut water (Jayanti, Rai, Dasgupta, & De, 2010; Sucupira et al., 2017).

* Corresponding author.
E-mail addresses: rmiranda@pq.cnpq.br, rmiranda@ufc.br (M.R.A.d. Miranda).

https://doi.org/10.1016/j.foodchem.2020.126874
Received 2 August 2019; Received in revised form 5 February 2020; Accepted 19 April 2020
Available online 21 April 2020

Thus, this work aimed to evaluate the chemical composition of tender green coconut water subjected to ultra-high temperature (UHT) sterilization by using ^{1}HNMR, UPLC-HRMS, GC–MS and chemometrics in order to identify chemical compounds that could be associated to pink color development from thermal-processing, in comparison to untreated tender water.

2. Experimental

2.1. Sampling

Immature green-colored coconuts (*Cocos nucifera* L.) with 6 to 7 months of development and harvested in Ceará state, Brazil, were initially rinsed in tap water and sanitized for 15 min in chlorinated water (100 mg·L^{-1} of sodium hypochlorite). For tender control sample, water was collected, filtered and stored at −17 ± 2 °C until analysis. Meanwhile, thermal processing of water was performed as reported by Sucupira et al. (2017). After filtration, samples were subjected to 110 °C with retention time of 8 s using an tubular heat exchanger (Armfield model FT74™, Armfield Inc, USA), cooled with chiller Armfield FT63, filled under aseptic conditions in 210 mL glass bottles that were closed with plastic screw cap. Previously, bottles were sterilized with 0.5% peracetic acid and rinsed with sterile water. After processing, coconut water was kept at room temperature until color turned to pink (same time of storage for all processed samples) and then, was stored at −17 ± 2 °C, until analyses. Thus, the experiment consisted of two treatments, control tender and thermally processed water with three biological repetitions, each.

2.2. UPLC-HRMS analysis

2.2.1. Extraction procedure

The solid phase extractions (SPE) of coconut water samples were performed in octadecylsilane (C18; 500 mg, 6 mL) reverse phase cartridges (Supelco™, Supelclean ENVI™, 18.58 μm, 58 Å; Sigma, USA). Initially, the C18 cartridges were inserted into the manifold coupled to a vacuum pump and conditioned with 3 mL of HPLC grade methanol followed by addition of 3 mL of water. Subsequently, 25 mL of coconut water was added to each cartridge. Clean up was done by applying 3 mL of water to eliminate sugars and amino acids from the extract. Finally, those compounds of interest were eluted by the addition of 5 mL of elution solvent 100% methanol HPLC grade. The removal of methanol was made on R-215 rotary evaporator (Buchi, Switzerland) at 40 °C and rotation of 20 rpm, and SPE was performed in triplicate.

2.2.2. Analysis

Ultra-Performance Liquid Chromatography Untargeted Mixed-Mode Tandem Mass Spectrometry (UPLC-HRMS) analysis was performed on an Acquity® UPLC system (Waters Co., USA) coupled with a Quadrupole Time-of-flight (Q-TOF) system (Waters, USA). A Waters Acquity™ UPLC Ethylene Bridged Hybrid (BEH) column (150 × 2.1 mm, 1.7 μm) was used with temperature set at 40 °C. Mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient used consisted: (0–15) min, 2–95% B; (15.01–17.0) min, 100% B; (17.01–19.01) min, 2% B, with flow rate of 0.400 mL·min^{-1}. Samples were dissolved in the water/methanol ratio (1:1) at 1 mg·mL^{-1} concentration, filtered through Millipore™ Teflon membranes with 0.22 μm pores, and the injection volume was 5 μL. Analysis used electrospray ionization (ESI) interface in negative and positive ion modes acquired from 110 to 1180 Da. Source temperature was 120 °C, desolvation temperature was 350 °C and desolvation gas flow of 500 L·h^{-1}. Leucine enkephaline was used as lock mass. The acquisition mode was MS^{E} and the instrument controlled by Masslynx 4.1 software (Waters Co., USA). Analyzes were performed in triplicate.

2.2.3. Chemometric analysis of UPLC-HRMS dataset

Unsupervised chemometric analysis by Principal Component Analysis (PCA) was performed using both matrices acquired in negative and positive ionization modes, in triplicate. Program Partial Least Squares (PLS) Toolbox™ (version 8.6.2 Eigenvector Research Inc., USA) was used to handle the multivariate data and analyses were performed using chromatograms region between 0.7 and 7.5 min for negative ionization mode, and 2.0 and 7.8 min for positive ionization mode, resulting in two numerical matrices with dimensionalities of 12,438 (18 samples × 691 variables) and 11,664 (18 samples × 648 variables).

The PCA was performed to determine the relationship between coconut water treatments regarding their secondary metabolites, with 95% of confidence level. Pretreatment of variables was performed with baseline correction (linear fit algorithm) and normalization, and samples were mean-centered, once this pretreatment provided better differences between coconut water treatments.

2.3. GC–MS analysis

2.3.1. Extraction and derivatization

Total lipids extracted from coconut water were analyzed as described by Bligh and Dyer (1959) with modifications. Water (300 mL) and 290 mL of chloroform were mixed in separating funnel, stirred vigorously and allowed to stand to liquid–liquid partition equilibrium. Chloroform, in the lower phase of separating funnel, was collected and the aqueous phase was re-used for further lipid extraction using the same volume of chloroform. The recovered chloroform was evaporated at 40 °C on rotary evaporator R-215 (Buchi, Switzerland).

The extraction procedure was performed in triplicate for each treatment. The derivatization of the fatty acids into volatile organic compounds (VOC) was performed according to Lutz- (2008). In a screw-capped glass tube, 20 mg of lipids were solubilized with 3 mL of GC-grade hexane. Then, 4 mL of methanolic solution with 0.5 M sodium hydroxide (NaOH) were added to the tube, closed and kept in a water-bath (65–70 °C) until complete dissolution of the fat globules (approximately for 4 min). After heating, the tube was cooled in running water and 5 mL of the esterifying solution (10 g of ammonium chloride, NH_4Cl dissolved in 300 mL of methanol and 15 mL of H_2SO_4) was added. After closing tube, the mixture was vortexed for 30 s, kept in water bath at 65–70 °C for 5 min, then cooled under running water. Subsequently, 4 mL of aqueous solution of 36% sodium chloride (NaCl) were added and vortexed for 30 s, followed by addition of 3 mL of GC-grade hexane and vortexing. For recovery of esters, the mixture was placed in a separating funnel to separate the aqueous and organic phases, the organic phase containing the esters was removed with an automatic micropipette and dried to remove hexane, then reconstituted with GC-grade hexane for the GC. Biological triplicates were performed for each coconut water.

2.3.2. Analysis

Samples were analyzed in Gas Chromatograph and Mass Spectrometer (GC–MS) (model 5977A Agilent Tech.® Inc., USA) equipped with a HP-5MS (Agilent®) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness) connected to a quadrupole detector operating in the electron impact (EI) mode at 70 eV with a scan mass range of 35–500 *m/z*. Helium was used as carrier gas at 1 mL.min^{-1}. The injector and the interface temperatures were 250 and 280 °C, respectively, in split mode (1:30). The temperature ramp was: 35 °C, increased to 180 °C at 15 °C min^{-1}, to 250 °C at 5 °C min^{-1}, and final temperature (250 °C) was held for 10 min. The linear retention indexes (LRI) were obtained using a standard solution of C_7–C_{30} saturated alkanes (Supelco, USA). For tentative identification, mass spectra were compared with the literature, with the USA National Institute of Standards and Technology (NIST) mass spectral library as well as spectral data and LRI provided by Adams (2007).

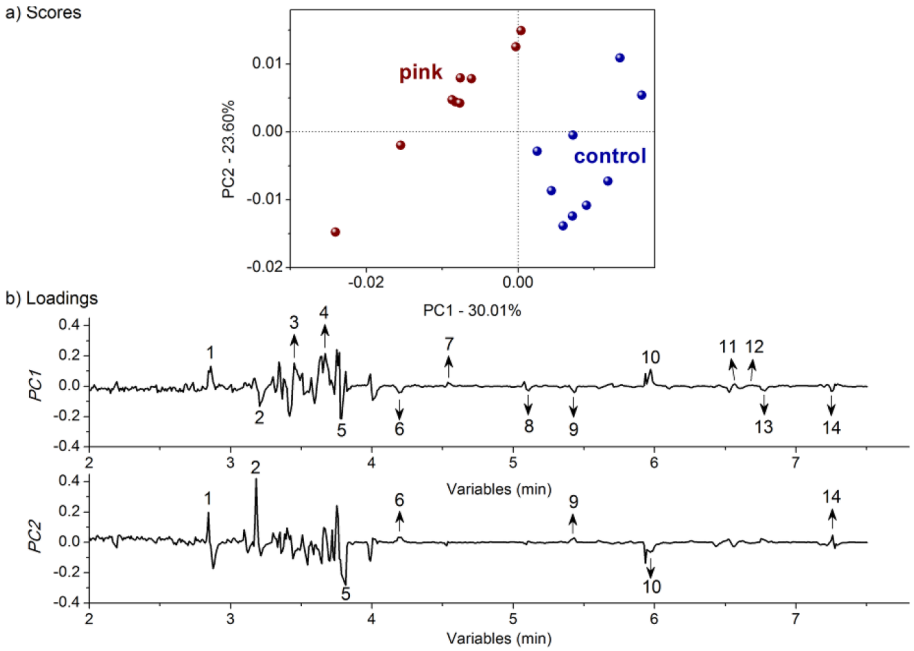

Fig. 1. Two-dimensional space plot for PC1 × PC2 scores coordinate system of the coconut water under negative ionization mode (1a) and relevant loadings plotted in line form (b), respectively.

2.3.3. Chemometric analysis of GC–MS dataset

The same program and pretreatments applied for chemometric evaluation of UPLC-HRMS datasets were applied to GC–MS dataset. The region of the chromatograms between 10 and 18 min was used for the analysis, resulting in a numerical matrix with dimensionality of 8976 (6 samples × 1496 variables).

2.4. NMR analysis

Samples were prepared as aliquots of coconut water (165 μL) were mixed with 400 μL of deutered water (D_2O, 98%), 35 μL de D_2O containing 1% of sodium-3-trimethylsilylpropionate (TMSP-2,2,3,3-d4 98% purity) with 25 mM of EDTA, and transferred to 5 mm NMR tubes. EDTA was added to minimize the ionic strength effect on frequency shifts in the NMR spectra

Analyses were performed on nuclear magnetic resonance (NMR) spectrometer (600-MHz Agilent Tech.® Inc., USA) equipped with a 5 mm (^{1}H-^{19}F/^{15}N-^{31}P) inverse detection One Probe™ with actively shielded z-gradient. The PRESAT pulse sequence was used to non-deuterated water suppression at chemical shift δ 4.85. The proton (^{1}H) NMR spectra were acquired under quantitative parameters, with 32 free induction decays (FID), 64 k of time domain points with a spectral window of 20.0 ppm, acquisition time of 5.0 s, and a relaxation delay of 15.0 s, all after pulses calibration. Spectra were processed using zero filling to 64 k points, phased manually and referenced using TMSP-d_4 at δ 0.0 as internal reference, and temperature was controlled at 298 K.

Two-dimensional (2D) NMR analyses were performed using the standard spectrometer library pulse sequences. ^{1}H–^{1}H *g*COSY (gradient correlation spectroscopy) experiments were obtained with spectral width of 7,267.4 Hz in both dimensions; 1 k × 200 data matrix; 16 scans per t1 increment and relaxation delay of 1.0 s. One-bond ^{1}H–^{13}C *g*HSQC (gradient heteronuclear single quantum coherence) experiments were acquired with an evolution delay of 3.425 ms for an average ^1J (C,H) of 146 Hz; 1 k × 200 data matrix; 32 scans per t1 increment; spectral widths of 9,615.4 Hz in f2 and 30,165.9 Hz in f1 and relaxation delay of 1.0 s. Long-range ^{1}H–^{13}C *g*HMBC (gradiente heteronuclear multiple bond coherence) experiments were recorded with an evolution delay of 62.5 ms for ^{LR}J(C,H) of 8 Hz; 1 k × 200 data matrix; 64 scans per t1 increment; spectral width 9,615.4 Hz in f2 and 36,199.1 Hz in f1 and relaxation delay of 1.0 s.

2.4.1. Chemometric analysis of NMR dataset

The same chemometric software and pretreatments applied to evaluate the UPLC-HRMS datasets were applied to ^{1}H NMR dataset. The spectral region between δ 0.8 and 9.0 was selected, which resulted in a numerical matrix with dimensionality of 150,804 (18 samples × 8378 variables into each spectrum). However, in order to enhance the chemical variability among the samples based on processing influence, the matrix was decomposed by Partial Least Squares – Discriminant Analysis (PLS-DA) method using the Simplified PLS (SIMPLS) algorithm, and the samples were clustered as control or thermally processed.

3. Results and discussion

Usually unsupervised multivariate analyses are applied to untargeted exploration of complexes food matrices of data to observe variations and relationships between coconut water and its composition (Sucupira et al., 2017). Therefore, PCA was performed to evaluate the coconut water variability under different processing condition, using different analytical techniques.

3.1. Metabolites assayed by UPLC-HRMS

PCA was applied to reduce the dimensionality of the original data in two PC that enabled to discriminate and classify samples and thus, identify metabolites responsible for the differences among treatments. Figs. 1 and 2 illustrate the PCA results from UPLC-HRMS datasets of the coconut water analyzed under negative and positive ionization mode, respectively, while Tables 1 and 2 describe the respective parameters for characterization of the relevant compounds of secondary metabolism, in chemometric evaluation.

According to negative ionization mode, PC1 × PC2 scores show tendency of separation of samples with 49.3% of total variance (Fig. 1a), and PC1 was the main axis related to coconut water separation based on processing. The loading (Fig. 1b) highlighted the greater relevance of compounds represented by numbers 2, 5 and 6 in thermally processed pink water, while compounds 1, 3, 4, and 10 were

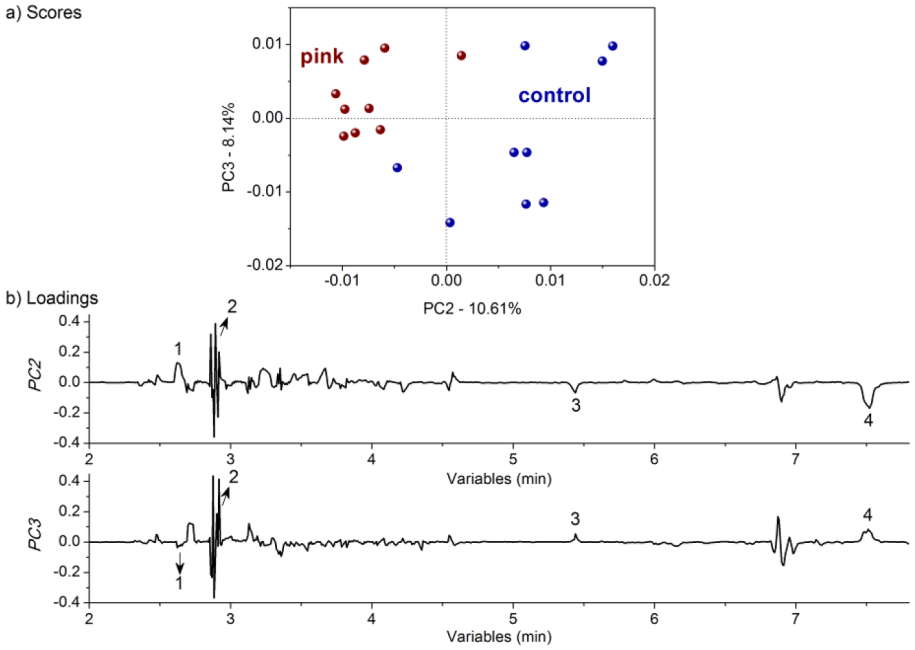

Fig. 2. Two-dimensional space plot for PC1 × PC2 scores coordinate system of the coconut water under positive ionization mode (2a), and relevant loadings plotted in line form (b), respectively.

more relevant in control water. Table 1 shows that compound 1 is cytokinin *trans*-zeatin riboside, a naturally occurring hormone involved with regulation of plant growth and development that was previously reported in coconut water (Ge, Yong, Tan, Yang, & Ong, 2004; Kobayashi et al., 1995) and in coconut milk by Van Staden and Drewes (1975). Compounds 2, 3 and 6 are respectively a trimer, dimer and dimer (type A) of procyanidin identified in thermally processed (2 and 6) and control (3) water. These phenolic compounds with antioxidant, anti-inflammatory and anticancer activities were observed in inflorescence of *Cocos nucifera* L. and their monomers, (+)-catechin and (−)-epicatechin were quantified in coconut water (Chang & Wu, 2011; Padumadasa, Dharmadana, & Abeysekera, 2016). Compound 6 is procyanidin dimer with A-type ether bond, C2-O-C7, besides the C4-C8 bond, and compound 2 is B-type procyanidin composed of three subunits linked by C4-C8 bonds.

Trimeric and A-dimeric procyanidins, identified in pink-colored thermally processed coconut water, are oligomeric procyanidins constituted of flavan-3-ols units that act as precursors of anthocyanins, pigments responsible for red to blueish color. Moreover, colorless procyanidins may also enhance the red color of anthocyanin solutions by preferentially interacting (ð- stacking) with the planar chromophore of the flavylium form, thus increasing its concentration (Malien-Aubert, Dangles, & Amiot, 2002; Reichel, Carle, Sruamsiri, & Neidhart, 2011). These authors reported that increase in polymeric degree improves the color stability.

Compounds 4 e 5 represent caffeoylshikimic acid, which is derived from caffeic acid and an intermediate of the synthesis of polymeric phenolics in plant cells, found in tender and thermally processed coconut water, respectively. Caffeoylshikimic acid detected in young coconut mesocarp has been associated to plant defense due to antioxidant and antimicrobial properties (Chakraborty & Mitra, 2008). Moreover, compounds 4 (caffeoylshikimic acid) and 11 (dicaffeoyl quinic acid) identified in control water, have a dioxygenated phenylpropanoid structure and can act as substrates of browning-associated enzyme polyphenoloxidase (PPO). Also relevant in control water, compound 10 is an oxidized fatty acid trihydroxy-octadecenoic acid, which has been associated with wound signals that triggers phenylalanine ammonia lyase (PAL) activity through jasmonic acid pathway, thus stimulating phenolic biosynthesis (García, García-Villalba, Gil, & Tomas-Barberan, 2017). Therefore, metabolites caffeoylshikimic acid (4), trihydroxy-octadecenoic acid (10) and dicaffeoyl quinic acid (11) mainly present in control water could act as substrates or signals triggering color reactions after thermal processing. Recently, García, Gil, and Tomas-Barberan (2018) confirmed the involvement of metabolites caffeoylquinic acid and trihydroxy-octadecenoic acid as biomarkers of pink-color development in fresh-cut lettuce. Although, compounds 8, 9, 13 and 14 were not identified, they accumulated only in thermally processed water, thus could be involved in color changes.

According to positive ionization mode, PC2 × PC3 scores show separation of the samples with 29.0% of the total variance (Fig. 2a). Both axes PC2 and PC3 were related to separation of coconut water based on processing, thus compounds represented by numbers 1 and 2 were relevant in control samples, and compounds 3 and 4 were relevant in thermally processed pink water (Fig. 2b). Table 2 shows compounds 1 and 2 are cytokinins *trans*-zeatin derivative and *trans*-zeatin riboside (in negative mode), respectively, while compounds 3 and 4 are unknown in literature.

3.2. Evaluation by GC–MS

Fig. 3 presents the scores (a) and relevant loadings (b) with fatty acids derivatized into volatile organic compounds (VOC) from coconut water. PC1 was the main axis related to coconut water separation based on processing and loadings showed that lauric (C12:0), myristic (C14:0), and elaidic (C18:1) acids were abundant in control, while palmitic (C16:0) and stearic (C18:0) acids levels were higher in thermally processed pink water. Table 3 describes these relevant VOC with the respective retention time (RT), linear retention index (LRI), major *m/z* ratio, and percentage of match.

Loadings showed that lauric, myristic, and elaidic acids content declined with the processing, while palmitic and stearic acids increased in relevance. Previously, Fonseca, Bizerra, De Souza, Monte, De Oliveira, De Mattos, Cordell, Braz-Filho, and Lemos (2009) and Santoso et al. (1996) reported the presence of long-chain fatty acids such as palmitic, myristic, elaidic and stearic acids in water of green dwarf coconut. Once the coconut is opened, there is an increase in production of free radicals as reactive oxygen species (ROS), which oxidize plant cell components especially polyunsaturated fatty acids as elaidic acid,

Table 1
Tentative peak (#) assignment identified by UPLC-HRMS in negative ion mode tentative of compounds from secondary metabolism of control and thermally processed coconut water. * Most relevant compounds in pink-colored thermally processed samples.

#	RT min	$[M-H]^-$obs.	$[M-H]^-$calc.	Product ion	Empirical formula	Ppm error	Compounds	References
1	2.86	1100.3920	1100.3894	968.3441; 836.3017; 806.2991; 644.2291; 512.1919	$C_{43}H_{66}N_5O_{28}$	2.4	14-O-{3-O-[β-D-galactopyranosy1-(1 → 2)-α-D-galactopyranosyl-(1 → 3)-α-L-arabinofuranosyl] −4-O-(α-L-arabinofuranosyl) -β-D-galactopyranosyl}-*trans*-zeatin riboside	Kobayashi et al. (1995)
2*	3.19	865.1958	865.1980	739.1705; 695.1409; 577.1321; 425.0870; 287.0544	$C_{45}H_{37}O_{18}$	−2.5	Procyanidin trimer	Hanhineva et al. (2008)
3	3.47	577.1336	577.1346	451.1045; 425.0854; 407.0735; 289.0669; 245.0786; 125.0212	$C_{30}H_{25}O_{12}$	−1.7	Procyanidin dimer	Hanhineva et al. (2008)
4	3.68	335.0708	335.0708	179.0325; 161.0212; 135.0418	$C_{23}H_{11}O_3$	0.0	Caffeoylshikimic acid	Kang, Price, Ashton, Tapsell, and Johnson (2016)
5*	3.77	335.0718	335.0708	179.0276; 161.0210; 135.0404	$C_{23}H_{11}O_3$	3.0	Caffeoylshikimic acid	Kang et al. (2016)
6*	4.20	575.1179	575.1190	539.0944; 449.0854; 423.0820; 407.0853; 289.0759; 285.0396	$C_{30}H_{23}O_{12}$	−1.9	Procyanidin dimer (type A)	Zang and Zhu (2015)
7	4.53	461.1075	461.1084	341.0847; 323.0992; 299.0511; 284.0263	$C_{22}H_{21}O_{11}$	−2.0	Chrysoeriol hexoside I	Kang et al. (2016)
8*	5.07	343.1539	343.1545	255.1743	$C_{20}H_{23}O_5$	−1.7	Unknown	–
9*	5.43	198.0784	198.0780	–	$C_{10}H_8O_5$	2.0	Unknown	–
10	5.97	329.2313	329.2328	311.2167; 293.2081; 229.1416; 211.1310; 171.0996	$C_{18}H_{33}O_5$	−4.6	Trihydroxy-octadecenoic acid	Llorent-Martínez et al. (2017)
11	6.52	515.2451	515.2492	353.2064; 209.1537; 125.0789	$C_{25}H_{39}O_{11}$	−8.0	Dicaffeoyl quinic acid	Farag, Weigend, Luebert, Brokamp, and Wessjohann (2013).

(*continued on next page*)

Table 1 (*continued*)

#	RT min	$[M-H]^-$ obs.	$[M-H]^-$ calc.	Product ion	Empirical formula	Ppm error	Compounds	References
12	6.62	503.3389	503.3373	217.1215	$C_{30}H_{47}O_6$	3.2	(+)-Arjungenin	Zhou et al. (2018).
13*	6.77	515.2455	515.2434	426.9656; 406.9587; 341.1802; 315.0395; 241.0134; 199.1682	$C_{32}H_{35}O_6$	4.1	Unknown	–
14*	7.27	236.1015	236.1008	221.1506; 220.1449; 192.1133; 177.0881	$C_7H_{10}N_9O$	3.0	Unknown	–

Table 2
Tentative peak (#) assignment identified by UPLC-HRMS in positive ion mode of the compounds from secondary metabolism of control and thermally processed coconut water. *Most relevant compounds in pink-colored thermally processed samples.

#	RT (min)	$[M-H]^+$ obs.	$[M-H]^+$ calc.	Prod. ion	Empirical formula	ppm error	Compounds	References
1	2.63	970.5812	970.5811	838.4927; 808.5039; 646.4130; 514.3276; 220.1659	$C_{44}H_{84}N_5O_{18}$	0.1	*trans*-Zeatin derivative	Zhao et al. (2013)
2	2.90	1102.4089	1102.4051	970.5733; 808.4877; 646.3964; 514.3232; 352.2343; 220.1646	$C_{43}H_{68}N_5O_{28}$	3.4	14-O-{3-O-[β-D-galactopyranosy1-(1 → 2)-α-D-galactopyranosyl-(1 → 3)-α-L-arabinofuranosyl]-4-O-(α-L-arabinofuranosyl) -β-D-galactopyranosyl}-*trans*-zeatin riboside	Kobayashi et al. (1995)
3*	5.44	200.0932	200.0936	–	$C_{10}H_{10}N_5$	−2.0	Unknown	–
4*	7.52	475.2904	475.2907	431.2616; 387.2361; 171.1388	$C_{24}H_{43}O_9$	−0.6	Unknown	–

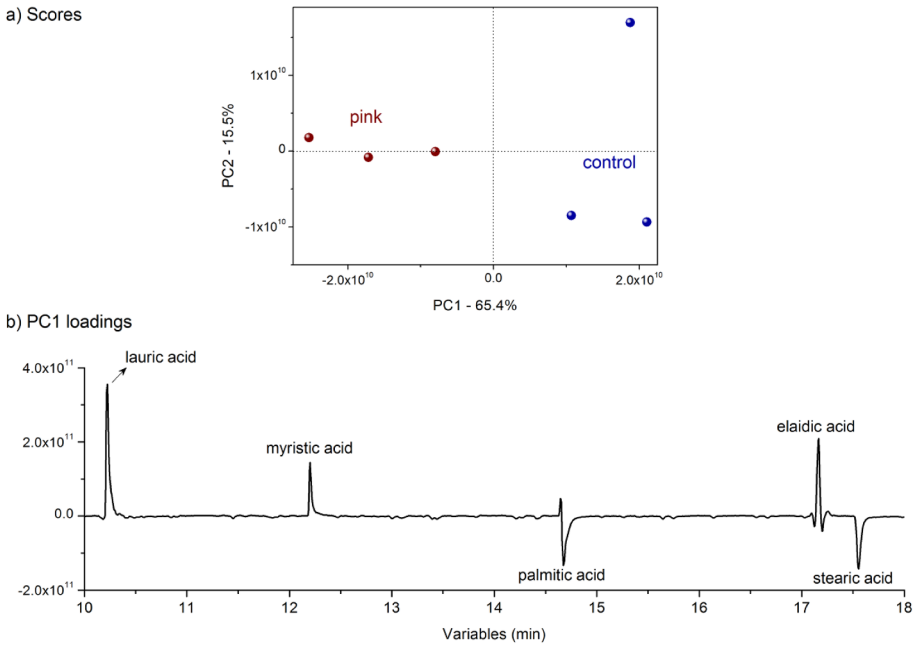

Fig. 3. Two-dimensional space plot for PC1 × PC2 scores coordinate system of the coconut water (a) and relevant loading plotted in lines form (b).

therefore, explaining its decline after processing (Evans & Halliwell, 2001). Moreover, the increase in saturated long-chain fatty acids as palmitic (C16:0) and stearic acids (C18:0) in thermally processed coconut water can be associated with resistance to oxidative reactions, as observed in coconut oil (Yousefi, Nateghi, & Rezaee, 2013).

3.3. Evaluation by NMR

Due to great number of identified compounds of the primary metabolism and the inherent similarity between the control and thermally processed pink coconut water, two supervised chemometric approaches by PLS-DA were developed in order to maximize the differences between treatments, one for each relevant region into the ^{1}H NMR spectra, separately. The classification results from the aliphatic region between the chemical shifts δ 0.8 and 3.0 are illustrated in Fig. 4a (scores) and 4b (relevant loadings); and from the carbinolic region between δ 3.0 and 5.5 in Fig. 4c (scores) and 4d (loadings). In both analyses, control samples were located at negative scores of PC1, while thermally processed pink samples at positive scores of the same PC. Based on the LV1 loadings, the most relevant compounds for discrimination between treatments were α-glucose, β-glucose, fructose, sucrose, ethanol, valine, lactic, acetic, and malic acids, which decreased with processing. The ^{1}H NMR chemical shifts are presented in Supplementary Material. Formic acid was the only compound identified in aldehydic and aromatic chemical shifts (δ 6.0–9.3), at δ 8.47, meanwhile ethanol is not a contaminant of processing once its presence also was verified in control coconut water.

4. Conclusions

Based on the analyses performed on tender and thermally processed pink-colored coconut water, NMR results showed primary metabolites decreased with processing, mainly fructose and α and β glucoses. GC–MS analyses showed that long-chain saturated palmitic and stearic acids accumulated in thermally processed coconut water, probably as result of oxidative reactions. However, UPLC-HRMS analyses pointed out a greater difference between treatments regarding the secondary metabolites. Thereby, procyanidin A-type dimer and trimer, in negative ionization mode, together with others unidentified compounds in negative (8, 9, 13 and 14) and positive (3,4) ionization modes were most relevant in thermally processed coconut water. Based on these results, it could be inferred that oligomeric procyanidins as A-type dimer and trimer could be associated with pink color development in coconut water as they are biosynthetic precursors of anthocyanin pigments and/or by enhancing color stability of anthocyanin solutions.

Thus, pink color development during storage of coconut water depends on postharvest conservation factors as thermal processing that may lead to accumulation of metabolites associated with color development. Although, pink coconut water is visually undesirable from consumeŕs point of view, the observed increase in phenolic content may influence its health promoting properties. Thus, our results show that integrating untargeted metabolomics strategies from data acquisition to process interpretation for the selection of chemical markers can be a good way to solve a technological problem as that of coconut water change of color. However, further studies are necessary to isolate the compounds responsible for pink color of thermally processed coconut

Table 3
Relevant volatile organic compounds detected in control and thermally processed coconut water, with respective retention times (RT), experimental and reference retention index (RI), major *m/z* peak, and percentage (%) of match. * Most relevant compounds in pink-colored thermally processed samples.

RT (min)	Compounds	RI* refer.	RI exp.	Major *m/z*	Match (%)	References
10.24	Lauric acid	1527	1532	74	94.5	Alissandrakis, Tarantilis, Harizanis, and Polissiou (2007)
12.20	Myristic acid	1727	1731	74	94.9	Radulović, Blagojević, and Palić (2010)
14.67	Palmitic acid*	1928.1	1929	74	95.7	Zeng et al. (2007)
17.17	Elaidic acid	2109.8	2106	55	94.7	Tret'yakov (2007)
17.55	Stearic acid*	2128	2130	74	93.2	Palmeira et al. (2004)

* RI – Retention index: retention times using *n*-alkenes series (C_7-C_{30}) converted in independent constants.

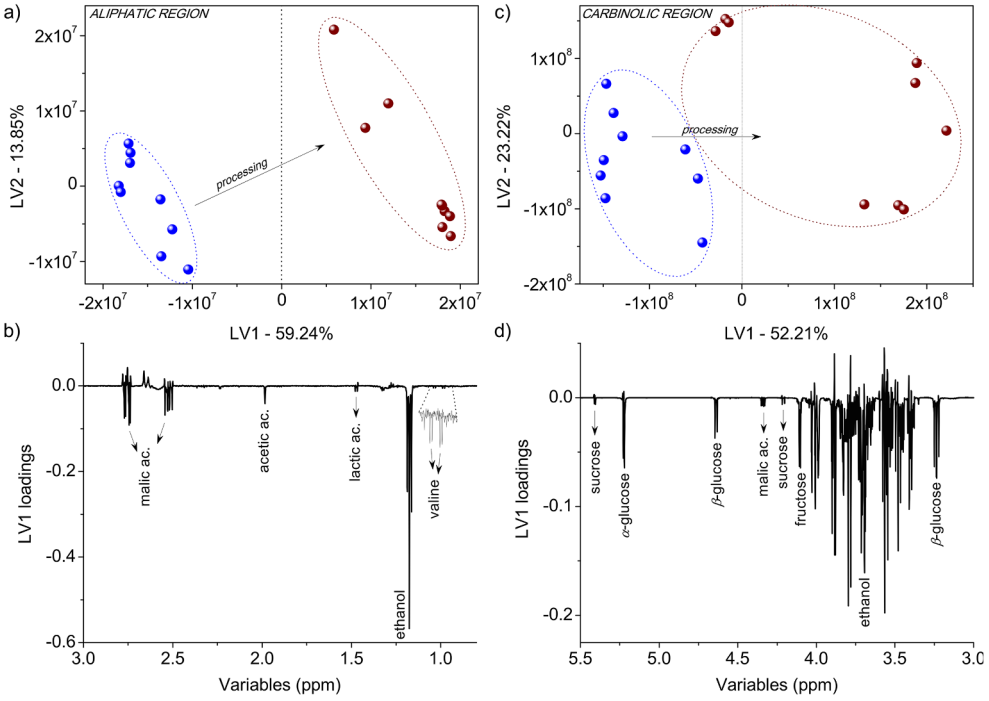

Fig. 4. The classification results from the aliphatic region between the chemical shifts δ 0.8 and 3.0 illustrated by scores (a) and the relevant loadings (b); and those from the carbinolic region between δ 3.0 and 5.5 by scores (c) and loadings (d).

water.

CRediT authorship contribution statement

Aline G. Cunha: Investigation, Formal analysis, Writing - original draft. **Elenilson G. Alves Filho:** Software, Data curation. **Lorena Mara A. Silva:** Formal analysis. **Paulo Riceli V. Ribeiro:** Formal analysis. **Tigressa Helena S. Rodrigues:** Formal analysis. **Edy S. de Brito:** Conceptualization, Writing - review & editing, Funding acquisition. **Maria Raquel A. de Miranda:** Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Instituto Nacional de Ciência e Tecnologia de Frutos Tropicais- INCT/CNPq, Brazil and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, Brazil for financial support, and FUNCAP for the scholarships (314737/2018-9) and (DCR-0024-01686.01.00/15).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126874.

References

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (4th ed.). Illinois: Allured Publishing Corporation.

de Aguiar, H. F., Yamashita, A. S., & Gut, J. A. W. (2012). Development of enzymic time-temperature integrators with rapid detection for evaluation of continuous HTST pasteurization processes. *LWT - Food Science and Technology, 47*(1), 110–116. https://doi.org/10.1016/j.lwt.2011.12.027.

Alissandrakis, E., Tarantilis, P. A., Harizanis, P. C., & Polissiou, M. (2007). Comparison of the volatile composition in thyme honeys from several origins in Greece. *Journal of Agricultural and Food Chemistry, 55*(20), 8152–8157. https://doi.org/10.1021/jf071442y.

Awuah, G. B., Ramaswamy, H. S., & Economides, A. (2007). Thermal processing and quality: Principles and overview. *Chemical Engineering and Processing: Process Intensification, 46*(6), 584–602. https://doi.org/10.1016/j.cep.2006.08.004.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917. https://doi.org/10.1139/o59-099.

Chakraborty, M., & Mitra, A. (2008). The antioxidant and antimicrobial properties of the methanolic extract from *Cocos nucifera* mesocarp. *Food Chemistry, 107*(3), 994–999. https://doi.org/10.1016/j.foodchem.2007.08.083.

Chang, C. L., & Wu, R. T. (2011). Quantification of (+)-catechin and (-)-epicatechin in coconut water by LC-MS. *Food Chemistry, 126*(2), 710–717. https://doi.org/10.1016/j.foodchem.2010.11.034.

Chen, W., Zhang, G., Chen, W., Zhongand, Q., & Chen, H. (2018). Metabolomic profiling of matured coconut water during post-harvest storage revealed discrimination and distinct changes in metabolites. *RSC Advances, 8*, 31396. https://doi.org/10.1039/c8ra04213f.

Damar, S., Balaban, M. O., & Sims, C. A. (2009). Continuous dense-phase CO_2 processing of a coconut water beverage. *Food Science & Technology, 44*, 666–673. https://doi.org/10.1111/j.1365-2621.2008.01784.x.

Debmandal, M., & Mandal, S. (2011). Coconut (Cocos nucifera L.: Arecaceae): In health promotion and disease prevention. *Asian Pacific Journal of Tropical Medicine, 4*(3), 241–247. https://doi.org/10.1016/S1995-7645(11)60078-3.

Evans, P., & Halliwell, B. (2001). Micronutrients: Oxidant/antioxidant status. *British Journal of Nutrition, 85*(S2), S67. https://doi.org/10.1049/BJN2000296.

Farag, M. A., Weigend, M., Luebert, F., Brokamp, G., & Wessjohann, L. A. (2013). Phytochemical, phylogenetic, and anti-inflammatory evaluation of 43 Urtica accessions (stinging nettle) based on UPLC-Q-TOF-MS metabolomic profiles. *Phytochemistry, 96*, 170–183. https://doi.org/10.1016/j.phytochem.2013.09.016.

Fonseca, A. M., Bizerra, A. M. C., De Souza, J. S. N., Monte, F. J. Q., De Oliveira, M. D. C. F., De Mattos, M. C., Cordell, G. A., Braz-Filho, R & Lemos, T. L. G. (2009). Constituents and antioxidant activity of two varieties of coconut water (Cocos nucifera L.). Brazilian Journal of Pharmacognosy, 19(1 B), 193–198. https://doi.org/10.1590/S0102-695X2009000200002.

García, C. J., García-Villalba, R., Gil, M. I., & Tomas-Barberan, F. A. (2017). LC-MS untargeted metabolomics to explain the signal metabolites inducing browning in fresh-cut lettuce. *Journal of Agricultural and Food Chemistry, 65*(22), 4526–4535. https://doi.org/10.1021/acs.jafc.7b01667.

García, C. J., Gil, M. I., & Tomas-Barberan, F. A. (2018). LC–MS untargeted metabolomics reveals early biomarkers to predict browning of fresh-cut lettuce. *Postharvest Biology and Technology, 146*, 9–17. https://doi.org/10.1016/j.postharvbio.2018.07.011.

Ge, L., Yong, J. W. H., Tan, S. N., Yang, X. H., & Ong, E. S. (2004). Analysis of some cytokinins in coconut (*Cocos nucifera* L.) water by micellar electrokinetic capillary chromatography after solid-phase extraction. *Journal of Chromatography A, 1048*(1),

119–126. https://doi.org/10.1016/j.chroma.2004.07.031.
Hanhineva, K., Rogachev, I., Kokko, H., Mintz-Oron, S., Venger, I., Kärenlampi, S., & Aharoni, A. (2008). Non-targeted analysis of spatial metabolite composition in strawberry (*Fragaria* × *ananassa*) flowers. *Phytochemistry, 69*(13), 2463–2481. https://doi.org/10.1016/j.phytochem.2008.07.009.
Instituto Adolfo Lutz- IAL. (2008). Procedimentos e determinações gerais. In: Métodos Físico-Químicos para Análises de Alimentos. (4th ed.). São Paulo: IMESP, (Chapter IV, 55-134p.).
Jayanti, V. K., Rai, P., Dasgupta, S., & De, S. (2010). Quantification of flux decline and design of ultrafiltration system for clarification of tender coconut water. *Journal of Food Process Engineering, 33*(1), 128–143. https://doi.org/10.1111/j.1745-4530.2008.00264.x.
Jirapong, C., Wongs-Aree, C., Noichinda, S., Uthairatanakij, A., & Kanlayanarat, S. (2015). Assessment of volatile and non-volatile organic compounds in the liquid endosperm of young 'Nam Hom' coconut (*Cocos nucifera* L.) at two stages of maturity. *The Journal of Horticultural Science and Biotechnology, 90*(5), 477–482. https://doi.org/10.1080/14620316.2015.11668703.
Kang, J., Price, W. E., Ashton, J., Tapsell, L. C., & Johnson, S. (2016). Identification and characterization of phenolic compounds in hydromethanolic extracts of sorghum wholegrains by LC-ESI-MS^n. *Food Chemistry, 211*, 215–226. https://doi.org/10.1016/j.foodchem.2016.05.052.
Kobayashi, H., Morisaki, N., Tago, Y., Hashimoto, Y., Iwasaki, S., Kawachi, E., & Shudo, K. (1995). Identification of a major cytokinin in coconut milk. *Experientia, 51*(11), 1081–1084. https://doi.org/10.1007/BF01946921.
Llorent-Martínez, E. J., Ortega-Barrales, P., Zengin, G., Mocan, A., Simirgiotis, M. J., Ceylan, R., & Aktumsek, A. (2017). Evaluation of antioxidant potential, enzyme inhibition activity and phenolic profile of *Lathyrus cicera* and *Lathyrus digitatus*: Potential sources of bioactive compounds for the food industry. *Food and Chemical Toxicology, 107*, 609–619. https://doi.org/10.1016/j.fct.2017.03.002.
Ma, Z., Ge, L., Lee, A. S. Y., Yong, J. W. H., Tan, S. N., & Ong, E. S. (2008). Simultaneous analysis of different classes of phytohormones in coconut (*Cocos nucifera* L.) water using high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry after solid-phase extraction. *Analytica Chimica Acta, 610*(2), 274–281. https://doi.org/10.1016/j.aca.2008.01.045.
Malien-Aubert, C., Dangles, O., & Amiot, M. J. (2002). Influence of procyanidins on the color stability of oenin solutions. *Journal of Agricultural and Food Chemistry, 50*(11), 3299–3305. https://doi.org/10.1021/jf011392b.
Murasaki-Aliberti, N. C., Silva, R. M. S., Gut, J. A. W., & Tadini, C. C. (2009). Thermal inactivation of polyphenoloxidase and peroxidase in green coconut (*Cocos nucifera*) water. *International Journal of Food Science and Technology, 44*, 2662–2668. https://doi:10.1111/j.1365-2621.2009.02100.x.
Padumadasa, C., Dharmadana, D., & Abeysekera, A. M. (2016). Distribution of progestogenic proanthocyanidins within the inflorescence of *Cocos nucifera* L. *Journal of Medicinal Plants Studies, 4*(4), 38–43.
Palmeira, S. F., de Moura, F. S., de Alves, V. L., Oliveira, F. M., de Bento, E. S., Conserva, L. M., & de Andrade, E. H. A. (2004). Neutral components from hexane extracts of Croton sellowii. *Flavour and Fragrance Journal, 19*(1), 69–71. https://doi.org/10.1002/ffj.1298.
Porto, E., Alves-Filho, E. G., Silva, L. M. A., Fonteles, T. V., Nascimento, R. B. R., Fernandes, F. A. N., & Rodrigues, S. (2020). Ozone and plasma processing effect on green coconut water. *Food Research International, 131*, 109000. https://doi.org/10.1016/j.foodres.2020.109000.
Prades, A., Assa, R. R. A., Dornier, M., Pain, J. P., & Boulanger, R. (2012). Characterisation of the volatile profile of coconut water from five varieties using an optimised HS-SPME-GC analysis. *Journal of the Science of Food and Agriculture, 92*(12), 2471–2478. https://doi.org/10.1002/jsfa.5655.
Prades, A., Dornier, M., Diop, N., & Pain, J.-P. (2012). Coconut water uses, composition and properties: A review. *Fruits, 67*(2), 87–107. https://doi.org/10.1051/fruits/2012002.
Radulović, N., Blagojević, P., & Palić, R. (2010). Comparative study of the leaf volatiles of *Arctostaphylos uva-ursi* (L.) Spreng. and *Vaccinium vitis-idaea* L. (Ericaceae). *Molecules, 15*(9), 6168–6185. https://doi.org/10.3390/molecules15096168.
Reichel, M., Carle, R., Sruamsiri, P., & Neidhart, S. (2011). Changes in flavonoids and nonphenolic pigments during on-tree maturation and postharvest pericarp browning of litchi (*Litchi chinensis* Sonn.) as shown by HPLC-MSn. *Journal of Agricultural and Food Chemistry, 59*(8), 3924–3939. https://doi.org/10.1021/jf104432r.
Rolle, R. (2007). *Good practice for the small-scale production of bottled coconut water.* Rome: FAO.
Santoso, U., Kubo, K., Ota, T., Tadokoro, T., & Maekawa, A. (1996). Nutrient composition of kopyor coconuts (*Cocos nucifera* L.). *Food Chemistry, 57*(2), 299–304. https://doi.org/10.1016/0308-8146(95)00237-5.
Sucupira, N. R., Alves Filho, E. G., Silva, L. M. A., de Brito, E. S., Wurlitzer, N. J., & Sousa, P. H. M. (2017). NMR spectroscopy and chemometrics to evaluate different processing of coconut water. *Food Chemistry, 216*, 217–224. https://doi.org/10.1016/j.foodchem.2016.08.035.
Tret'yakov, K., V. (2007). Retention data NIST mass spectrometry data center. http://webbook.nist.gov/cgi/inchi/InChI%3D1S/C13H26O2Si/c1-5-6-7-8-9-10-11-12-13%2814%291516%282,3%294/h5H,1,6-12H2,2-4H3. Accessed 29 December 2014.
Van Staden, J., & Drewes, S. E. (1975). Identification of zeatin and zeatin riboside in coconut milk. *Physiologia Plantarum, 34*(2), 106–109. https://doi.org/10.1111/j.1399-3054.1975.tb03801.x.
Yousefi, M., Nateghi, L., & Rezaee, K. (2013). Investigation of physicochemical properties, fatty acids profile and sterol content in Malaysian coconut and palm oil. *Annals of Bilogical Research, 4*(4), 214–219. http://refhub.elsevier.com/S0308-8146(17)30863-4/h0250.
Zang, S., & Zhu, M. J. (2015). Characterization of Polyphenolics in Grape Pomace Extracts Using ESI Q-TOF MS/MS. *Journal of Food Science & Nutrition, 1*(1), 1–10. https://doi.org/10.24966/FSN-1076/100001.
Zeng, Y. X., Zhao, C. X., Liang, Y. Z., Yang, H., Fang, H. Z., Yi, L. Z., & Zeng, Z. D. (2007). Comparative analysis of volatile components from Clematis species growing in China. *Analytica Chimica Acta, 595*(1–2 SPEC. ISS.), 328–339. https://doi.org/10.1016/j.aca.2006.12.022.
Zhao, H., Jiang, M., Liang, Q., Xie, C., Song, S., Wang, J., & Luo, G. (2013). Fragmentation pathway studies of several plant hormones using an electrospray ionization-quadrupole/time-of-flight mass spectrometer. *International Journal of Mass Spectrometry, 335*, 7–15. https://doi.org/10.1016/j.ijms.2012.10.011.
Zhou, J., Yi, H., Zhao, Z.-X., Shang, X.-Y., Zhu, M.-J., Kuang, G.-J., ... Zhang, L. (2018). Simultaneous qualitative and quantitative evaluation of Ilex kudingcha C. J. tseng by using UPLC and UHPLC-qTOF-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis, 155*, 15–26. https://doi.org/10.1016/j.jpba.2018.02.037.