Monitoring the physicochemical degradation of coconut water using ESI-FT-ICR MS

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A R T I C L E   I N F O

A B S T R A C T

Fresh and aged coconut water (CW) samples were introduced directly into the electrospray ionisation (ESI) source, and were combined with the Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) technique to characterise in situ chemical compounds produced during natural ageing (from 0 to 15 days) at room temperature (23 °C). The ESI-FT-ICR MS readings were acquired and the data were correlated to conventional methodologies: pH, total titratable acidity (TA), total soluble solids, microbial analyses, and ultraviolet visibility (UV–vis) spectroscopy analysis. In general, the pH and TA values changed after 3 days of storage making the CW unsuitable for consumption. The ESI(−)-FT-ICR data also showed a clear and evident change in the chemical profile of CW after 3 days of ageing in the m/z 150–250 and 350–450 regions. Initially, the relative intensity of the natural markers (the m/z 215 and 377 ions–sugar molecules) decreases as a function of ageing time, with the last marker disappearing after 3 days of ageing. New chemical species were then identified such as: citric (m/z 191), galacturonic (m/z 193), gluconic (m/z 195), and saccharic (m/z 209) acids. ESI(−)-FT-ICR MS is a powerful tool to predict the physicochemical properties of CW, such as the pH and TA, where species such as fructose, glucose, sucrose, and gluconic acid can be used as natural markers to monitor the quality of the fruits.

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1. Introduction

Coconut (*Cocos nucifera* L.) is a well-known tropical plant cultivated in equatorial and sub-equatorial areas to produce copra, the dried kernel that is later processed into oil. However, the coconut palm is also called the "tree of life" in Asia and can provide many other useful products. Coconut water (CW) is a traditional sweet and refreshing tropical drink that comes directly from the inner part of immature fruits. It is a clear liquid with good flavour and low calories, being highly valued and consumed in tropical areas of the world. Additionally, it is also used to restore electrolytes in cases of dehydration (Fontes, Ferreira, & Siqueira, 2002), such as inorganic salts containing ions of sodium, potassium, chloride, calcium, and magnesium, and complex organic molecules. These electrolytes are very important for homeostasis. CW is also a powerful source of ascorbic acid (vitamin C) and of the vitamin B complex, where a wide variability in its physicochemical composition can occur according to the stage of maturation, variation between species, and the soil components (Khan, Muti-Ur-Rehman, & Khan, 2003; Medeiros & Medeiros, 2012).

In Brazil, the consumption of CW has increased in the last few years, mainly because consumers are looking for natural and healthy products. There has recently been a rapid expansion of coconut palm plantations for the extraction of the water, with these plantations now occupying more than 57,000 ha in the Southeast, North, and Northeast regions of Brazil (Piló et al., 2009).

CW is initially sterile and remains aseptic until the fruit suffers from injuries that allow for the entry of microorganisms. The fruit should be harvested carefully to avoid mechanical damage. The harvested coconut can be kept for 15 days at room temperature without a trace of any obvious deterioration. After this time, fermentation begins in earnest and the CW is no longer fit for

consumption. However, if the water is removed from the fruit and stored at room temperature, it deteriorates in just a few days. This is because CW has an average pH of 5, and therefore microorganisms such as yeasts can grow easily in this natural medium (Prades, Ablan Assa, Dornier, Dor, & Boulanger, 2012; Sierra & Velasco, 1976). The chemical compounds that are related to the biological effects and physicochemical degradation of CW are not fully known. The physicochemical degradation can be related to the different forms of conservation and processing. However, to the best of our knowledge, there are no studies to determine the influence of these processes on the chemical and biochemical degradation mechanisms.

Currently, ultra-high resolution and accuracy mass spectrometry such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) allows for the identification of complex organic mixtures without the need for any prior extraction or separation steps. FT-ICR MS is applied in all the "omics" sciences (i.e. metabolomics, proteomics, and petroleomics), thus enabling molecular-level analyses of complex mixtures (Beens & Brinkman, 2000; Klein, Kim, Rodgers, & Marshall, 2006). Accurate mass measurements (Hsu, Hendrickson, Rodgers, McKenna, & Marshall, 2011; Savory et al., 2011) define the unique elemental composition (CcHhNnOoSs) from singly charged ions such as [M+H]$^+$, [M+Na]$^+$, [M+K]$^+$, [M−H]$^-$, and [M+Cl]$^-$, where M corresponds to a neutral molecule (Benassi et al., 2013; Colati et al., 2013; Cordeiro et al., 2013; Dalmaschio et al., 2014; Freitas et al., 2013; Haddad et al., 2012). Herein, we introduced CW samples directly into the electrospray ionisation (ESI) source, without prior separation, and linked this to the FT-ICR MS to characterise in situ chemical compounds produced during natural CW ageing (from 0 to 15 days) at room temperature (23 °C). The ESI-FT-ICR MS data were obtained and compared with conventional methodologies (pH, total titratable acidity (TA), total soluble solids (TSS), microbial analyses, and ultraviolet–visible (UV–vis) spectroscopy).

2. Experimental

2.1. Physicochemical degradation of CW

Three coconuts (*C. nucifera*) were purchased locally in Vitória City, Espírito Santo State, Brazil. Initially, each sample was cleaned by washing with soap and water followed by washing in an ethanol solution (70% v/v). The CW samples were then filtered using filter paper (125-mm porosity) and previously sterilized at room temperature (25 °C). Nine aliquots of 100 mL were collected as a function of ageing time (at 2, 4, and 6 h, and at 1, 3, 5, 7, 11, and 15 days) and stored at −20 °C, thus conserving their chemical properties. After the physicochemical properties of the aged CW samples were monitored using pH, TA, TSS (expressed in% and °Bx, respectively), microbial analyses (a global count of aerobic mesophilic heterotrophic microorganisms and molds), and chemical analyses such as ESI-FT-ICR MS and UV–vis spectroscopy.

2.2. ESI-FT-ICR MS

Briefly, the fresh and aged CW samples were acidified with 0.1% v/v of formic acid for ESI(+), and basified with 0.1% v/v of NH$_4$OH for ESI(−), and directly infused at a flow rate of 5 μL min^{-1} into the ESI source. The mass spectrometer (model 9.4 T Solarix, Bruker Daltonics, Bremen, Germany) was set to operate over a mass range of m/z 150–2000. The ESI source conditions were as follows: a nebulizer gas pressure of 0.5–1.0 bar, a capillary voltage of 2.5–3.5 kV, and a transfer capillary temperature of 250 °C. The ions were accumulated in the hexapolar collision cell with a time ranging from 1 to 5.10^{-3} s, followed by transport to the analyzer

cell (ICR) through the multipole ion-guide system (another hexapole). The time-of-flight in the hexapole was 0.7 ms. Each spectrum was acquired by accumulating 32 scans of time-domain transient signals in 4 mega-point time-domain data sets. All mass spectra were externally calibrated using arginine (for ESI(+)) and NaTFA (for ESI(−)) solutions (m/z from 200 to 2000). A resolving power, $m/\Delta m_{50\%}$ = 370–560,000 (in which $\Delta m_{50\%}$ is the full peak width at half-maximum peak height of $m/z \cong 400$), and a mass accuracy of <3 ppm provided the unambiguous molecular formula assignments for singly charged molecular ions. The mass spectra were acquired and processed using Data Analysis software (Bruker Daltonics, Bremen, Germany). The MS data were processed and the elemental compositions of the compounds were determined by measuring the m/z values. The proposed structures for each formula were assigned using the chemspider (www.chemspider.com) database. The aromaticity of each molecule can be deduced directly from its double bond equivalent (DBE) value according to Eq. (1):

$$DBE = c - h/2 + n/2 + 1 \tag{1}$$

where c, h, and n are the numbers of carbon, hydrogen, and nitrogen atoms, respectively, in the molecular formula.

2.3. Physicochemical assay

Fresh CW and its degradation products were monitored using pH, TA (%), and TSS (°Bx) analyses. The measurements of pH and TSS were performed using a pH meter (Metron® model 827) and a manual refractometer (Euromex® with readings that ranged from 0 to 32 °Bx, Ref. 103, and with a compensation temperature of 20 °C), respectively. TA measurements were performed using a 0.1 mol L^{-1} NaOH (Vetec Química Fina Ltda, Brazil) solution as the titrant. The results were expressed as grams of citric acid per 100 mL of sample. Finally, the TSS/TA ratio was calculated to evaluate the quality of CW. All analyses were done in triplicate and performed in accordance with methods described by Instituto Adolfo Lutz (Normas analíticas do Instituto Adolfo Lutz. IV-Métodos químicos e físicos para análise de alimentos, 2005).

2.4. Microbial analysis

The global count of aerobic mesophilic heterotrophic microorganisms and molds and yeasts was obtained for the frozen samples for 0, 1, 3, 5, 7, 11, and 15 days. Counts were determined by the surface-spreading method (Silva, Junqueira, & Silveira, 1997) employing 0.1 mL of sample solution prepared in plate count agar (35 °C per 24 h) and in Sabouraud agar for molds and yeasts (28 °C per 7 days). The analyses were performed in duplicate. After incubation, the colonies were observed and counted for each group, being expressed as a colony forming unit per mL of sample.

2.5. UV–vis analysis

CW samples were diluted (3.10^{-2}% v/v) and analysed using a UV–vis spectrophotometer (PerkinElmer, model Lambda 45) with the dual-beam mode. A total of 64 scans were undertaken and the spectra were recorded from 200 to 700 nm in absorbance mode with a resolution of 4 cm^{-1}.

3. Results and discussion

3.1. ESI(±)FT-ICR MS

ESI-FT-ICR mass spectra in both ionisation modes, ESI(+) and ESI(−), were acquired to evaluate the chemical profile of CW

samples as a function of ageing time. When analysing the initial ambient degradation conditions (an ageing time of between 0 and 6 h), no changes were observed in the chemical profiles of FT-ICR mass spectra. Fig. 1a and b show the typical ESI(+) (Fig. 1a) and ESI(−) FT-ICR (Fig. 1b) mass spectra of aged CW for a period of 6 h. For the ESI(+)-FT-ICR mass spectra (Fig. 1a), m/z 219.0266 (majority specie) and 399.0897 ions were detected as potassium adducts ($[C_6H_{12}O_6+K]^+$ and $[C_{12}H_{24}O_{12}+K]^+$ ions). Their molecular formulas correspond to fructose and/or glucose, and sucrose molecules, respectively. Additionally, the accurate m/z values (219.0266 and 399.0897) are also in agreement with the calculated m/z values (219.0265 and 399.0899), detected with mass deviations lower than 1 ppm (0.25 and 0.56 ppm, respectively). The DBEs of 1 and 2 for fructose/glucose and sucrose molecules indicate the presence of one (furan and/or pyran) and two (furan and pyran) rings, respectively. Therefore, accurate mass measurement can be used for a more robust identification of the chemical profile of the species present in these fruits.

For the ESI(−)-FT-ICR mass spectra (Fig. 1b), a similar chemical profile was observed in relation to the ESI(+) spectrum. Fructose/sucrose and sucrose molecules were detected as chlorine adducts: the m/z 215.0328 and 377.0858 ions corresponded to the chemical structures $[C_6H_{12}O_6+Cl]^-$ and $[C_{12}H_{24}O_{12}+Cl]^-$, respectively. Other molecules, such as aldobiuronic and D-glucopyranosylhexonic acids with the molecular formulas M = $C_{12}H_{20}O_{12}$ and $C_{12}H_{22}O_{12}$ were detected in both forms: the deprotonated and chlorine adduct ion $[M−H]^-$ of m/z 355.0883 and 357.1039, and the $[M+Cl]^-$ ion of m/z 391.0649 and 393.0805, respectively. Herein, the experimental isotopologue pattern of chlorine adducts is a characteristic that is due to the chlorine atom (the relative intensity of the m/z M+35Cl ion is 1/3 of that of m/z M+37Cl); see the inset of Fig. 1b that corresponds to the isotopologue pattern of the m/z 393.0805 ion. Generally, the abundant presence of potassium and chlorine adducts is commonly related to the high mineral concentrations present in the CW samples (Prades, Ablan Assa, Dornier, Dor, & Boulanger, 2012; Richter, Jesus, Muñoz, Lago, & Agnes, 2005; Thampan & Rethinam, 2004; Ferreira et al., 2009).

FT-ICR MS was then applied to evaluate the chemical profile of aged CW for a longer period of ageing time (from 1 to 15 days)

compared to that used in the shorter period (<24 h). It is well known that the high salt concentrations present in natural products can suppress chemical ionisation, mainly with ESI(+), due to the presence, simultaneously, of Na^+, Mg^{2+}, Mn^{4+}, Al^{3+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, and Cu^+ ions. Thus, the ESI(−) approach is more suitable for analysing the chemical profile of fruits.

The ESI(−)-FT-ICR mass spectra of the fresh and aged CW for a period of time ranging from 1 to 15 days are shown in Fig. 2. A clear and evident change in the chemical profile of CW was observed after 3 days of ageing in the m/z 150–250 and 350–450 regions. Initially, the relative intensity of the natural markers (the m/z 215 and 377 ions–sugar molecules) decreases as a function of ageing time, with the last marker disappearing after 3 days of ageing. New chemical species were then identified, and their chemical, structural, and molecular formulas, measured and theoretical m/z values, mass errors, and DBEs are shown in Table 1.

In the m/z 150–250 region, four acid species were detected: citric (m/z 191), galacturonic (m/z 193), gluconic (m/z 195), and saccharic (m/z 209) acids. Among them, gluconic acid was the most abundant. These species can be produced from hydrolysis reactions converting polysaccharides into oligosaccharides and monosaccharides, and disaccharides into monosaccharides, followed by oxidative degradation (Onda, Ochi, & Yanagisawa, 2011). In the 350–450 m/z region, other acid species of lower relative intensity were observed as m/z 369, 371, 373, 387, and 391 ions (all detected in their deprotonated form, $[M−H]^-$), and the 393 ion (which corresponds to the chlorine adduct, $[M+Cl]^-$). The m/z 425 and 429 ions were identified as $[M+K−2H]^-$, simultaneously. Note that the m/z 391 ion corresponds to a dimer of 2,3,4,5,6 penta-hidroxyhexanoic acid.

3.2. Physicochemical assay

Generally, the low pH and the high sugar concentrations in CW are factors that promote the development of yeasts in these beverages (Piló et al., 2009). Fig. 3a shows the variation in pH versus ageing time for CW: Initially, pH analyses show values of pH = 4.8–5.0 at up to 3 days of ageing. These values relate to the established values from the Agência Nacional de Vigilância Sanitária (ANVISA),
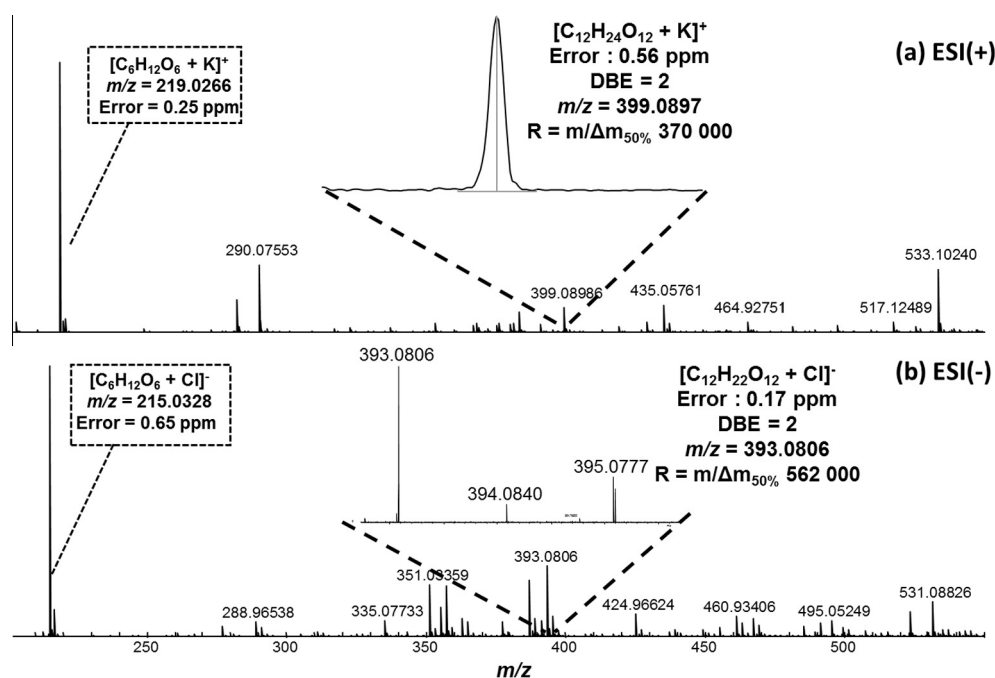

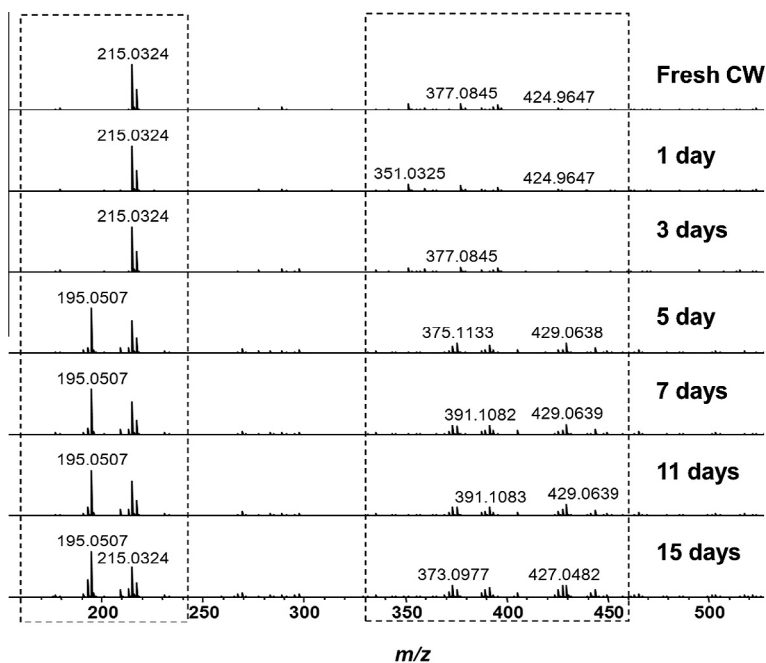**Fig. 1.** (a) ESI(+) and (b) ESI(−) FT-ICR mass spectra of aged CW over a period of 6 h.

Fig. 2. ESI(−)FT-ICR mass spectra of CW samples as a function of ageing time (days).

as they are between a pH of 4.3 and 6.0; thus, they are suitable for consumption (Brasil, 2002). After 3 days of degradation, a rapid decrease in the pH was observed (from $pH_{3days} = 4.8$ to $pH_{5days} = 3.3$), making the CW unsuitable for consumption.

A similar behaviour was observed when we monitored the TA values as a function of ageing time (Fig. 3c). Again, for the first few days of ageing (from 0 to 3 days), the TA values remain constant (0.0787–0.0820 g of citric acid per 100 mL of sample); levels that are in agreement with the Brazilian legislation that establishes maximum limits in the range of 0.02 g of citric acid per 100 mL of sample (Brasil, 2002). TA values increase from the fifth day of ageing and reach maximum levels of 0.3600 g of citric acid per 100 mL of sample at 15 days of ageing.

TSS measurements were used to monitor the sugar concentration present in CW. TSS versus ageing time shows variations after 7 days of opening and storing the CW (from 5 to 4.3 °Bx) (Fig. 3b). This is due to a reduction in the sugar concentration present, as observed from the ESI(−)-FT-ICR data. When the TSS/AT ratio was plotted (Fig. 3d) to evaluate the physicochemical quality of CW, a maximum plateau was reached, which was constant for 3 days of ageing (TSS/AT ratio of 61.72). This is indicative of good quality. In this case, a high sugar concentration was reached (Malézieux & Bartholomew, 2003). Finally, lower values were obtained immediately after 5 days of natural ageing and thus the CW became unsuitable for consumption.

In general, the pH, AT, TSS, and TSS/AT measurements are important parameters with which to assess the hygienic-sanitary quality of CW and to determine the physical and chemical characteristics of CW provided to the public in large Brazilian cities. All of the results were in good agreement with the ESI-FT-ICR MS data, which shows that the chemical profile of the CW greatly changed after 5 days of natural ageing (Fig. 2d).

The ESI(−)FT-ICR mass spectra are able to predict physicochemical parameters such as the pH and TA of CW samples from simple univariate linear calibration. The ratio of acid species (m/z 191, 193, and 195 ions) by sugar molecules (m/z 215 and 377 ions) versus pH and TA values (Fig. 4) were plotted, and the following equations 2 and 3 can be used to estimate the pH and TA values present in the CW samples, respectively:

$$y = 7.14x_1 - 0.57 \tag{2}$$

$$y = -1.31x_2 + 6.28 \tag{3}$$

where $y = (I191 + I193 + I195)/(I215 + I377)$, $x_1 = $ TA, and $x_2 = $ pH.

3.3. Microbial analysis

The global count of aerobic heterotrophic mesophyllic microorganisms and molds and yeasts was evaluated for fresh and aged CW samples in which the values reduced as a function of ageing time. This result is a direct indication that the standard growth curve of microorganisms in closed systems will only increase while nutrients are available in the environment that can be appropriated. In this case, CW obtained directly from fruits did not show any kind of microbial growth (Piló et al., 2009), thus suggesting that the counts obtained are probably due to contamination from other sources. However, the low pH and the high sugar concentrations in CW of good quality are factors that easily promote the development of yeasts in these beverages. Additionally, the numbers of aerobic heterotrophic mesophyllic microorganisms and molds and yeasts in CW obtained were similar to those reported in the literature (Piló et al., 2009).
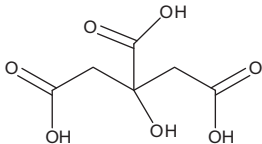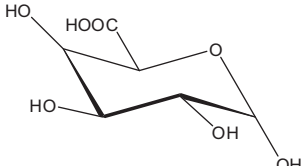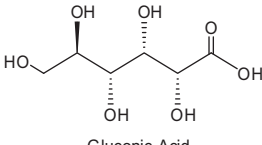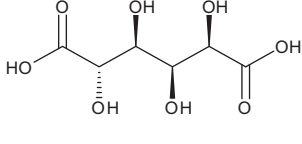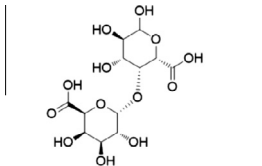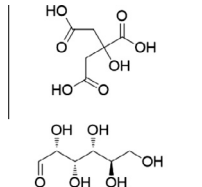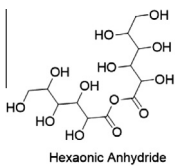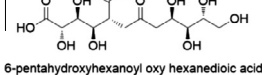
Although the results showed microbial growth in CW decreased over time, other authors have reported that the total aerobic counts in untreated CW increased after 6 weeks of storage, and that molds and yeasts showed no growth in the total period of 9 weeks of investigation (Damar, Balaban, & Sims, 2009). The decreasing microbial growth observed from the third to fifteenth days is due to an inverse tendency for the TA values, which means an acid accumulation medium that becomes unsuitable for microbial growth. Besides, the sugar concentration decreases as a function of storage time.

3.4. UV–vis analysis

From organoleptic analysis it is possible to identify the increasing turbidity in aged CW samples visually, beyond the change in colour, odour, and gas production (Tournas, Heeres, & Burgess, 2006).
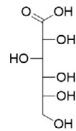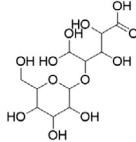
Table 1
Chemical species proposed from ESI(−)FT-ICR MS data for aged CW samples (from 3 to 15 days).

Molecular formula	$m/z_{Measured}$	$m/z_{Theoretical}$	Error (ppm)	DBE	Chemical structures
$[C_6H_8O_7-H]^-$	191.0197	191.0186	1.35	3	Citric Acid
$[C_6H_{10}O_7-H]^-$	193.0354	193.0343	1.56	2	Galacturonic Acid
$[C_6H_{12}O_7-H]^-$	195.0510	195.0499	1.45	1	Gluconic Acid
$[C_6H_{10}O_8-H]^-$	209.0303	209.0292	1.59	2	Saccharic Acid
$[C_{12}H_{18}O_{13}-H]^-$	369.0665	369.0675	2.70	4	Galactopyranuronosyl-D-galactopyranuronic
$[C_{12}H_{20}O_{13}-H]^-$	371.0821	371.0831	2.70	3	D-Glucose - 2-hydroxy-1,2,3-propanetricarboxylic acid
$[C_{12}H_{22}O_{13}-H]^-$	373.0978	373.0988	2.64	2	Hexaonic Anhydride
$[C_{12}H_{20}O_{14}-H]^-$	387.0770	387.0780	2.77	3	6-pentahydroxyhexanoyl oxy hexanedioic acid

(*continued on next page*)

Table 1 (continued)

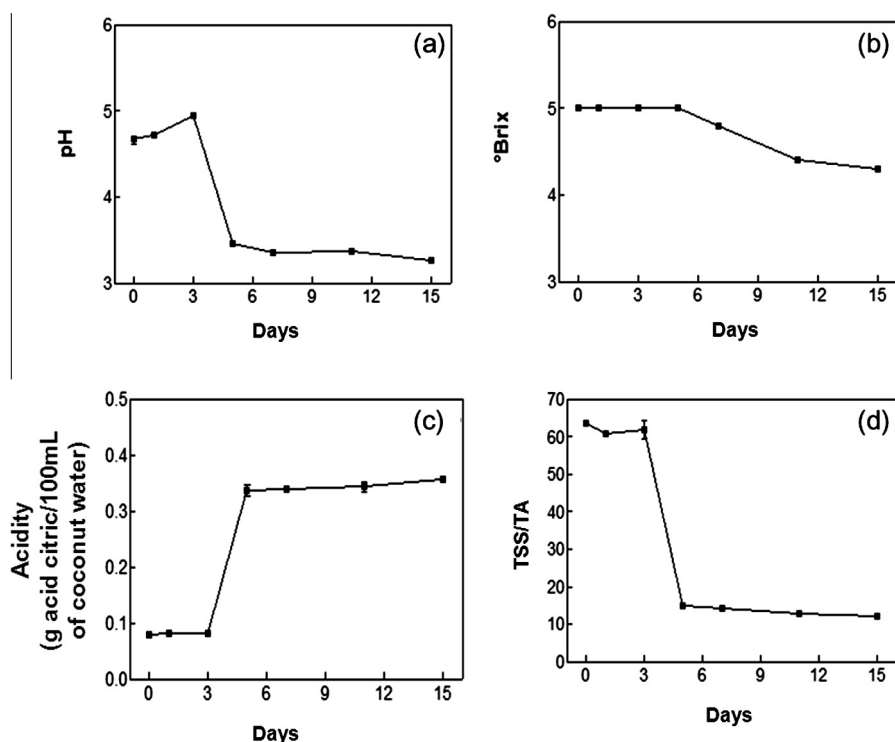| Molecular formula	$m/z_{Measured}$	$m/z_{Theoretical}$	Error (ppm)	DBE	Chemical structures
$[C_{12}H_{24}O_{14}-H]^-$	391.1083	391.1093	2.78	1	2,3,4,5,6-pentahydroxyhexanoic acid
$[C_{12}H_{22}O_{12}+Cl]^-$	393.0794	393.0805	2.78	1	4-O-Hexopyranosylhexonic acid
$[C_{12}H_{20}O_{14}+K-2H]^-$	425.0326	425.0339	3.04	3	
$[C_{12}H_{24}O_{14}+K-2H]^-$	429.0639	429.0652	3.08	1	

Fig. 3. Physicochemical assays of CW using conventional methodologies: (a) pH; (b) TSS; (c) acidity; and (d) TSS/TA ratio.

To evaluate the change in colour, UV–vis analyses were performed in the 200–700 nm region (Fig. 5). An increasing absorbance was observed in the 275–280 nm region as a function of ageing time and can be attributed to the degradation of proteins and sugar compounds, producing amino-acid molecules and acid species (Verza, Kreinecker, Reis, Henriques, & Ortega, 2007; Zaia, Zaia, & Lichtig, 1998). This last finding was confirmed with the ESI(−)-FT-ICR MS data (Fig. 2).

4. Conclusion and perspective

Ultra-high resolution and accuracy mass spectrometry such as Fourier transform ion cyclotron resonance mass spectrometry

(FT-ICR MS) is a powerful analytical technique in the chemical characterisation at molecular level of complex organic mixtures without the need for prior extraction or separation steps. FT-ICR MS allied to an electrospray ionisation (ESI) source enables the characterisation of in situ chemical compounds produced during the natural ageing of CW. For fresh CW, fructose/glucose (majority species) and sucrose molecules were identified as potassium and chlorine adducts ($[C_6H_{12}O_6+Cl]^-$, $[C_6H_{12}O_6+K]^+$, $[C_{12}H_{24}O_{12}+Cl]^-$, and $[C_{12}H_{24}O_{12}+K]^+$ ions of m/z 215, 219, 377, and 399). Other molecules, such as aldobiuronic and d-glucopyranosylhexonic acids with the molecular formula of M = $C_{12}H_{20}O_{12}$ and $C_{12}H_{22}O_{12}$ were also detected. The presence of chlorine in the molecular structure
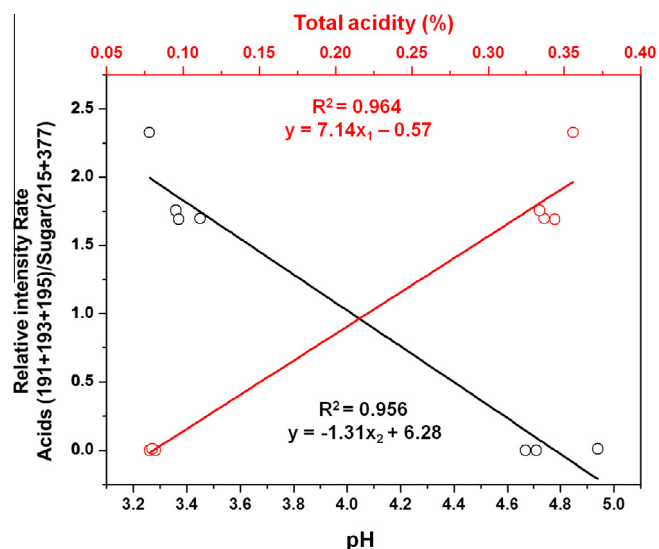
Fig. 4. Plot of (I191 + I193 + I195)/(I215 + I377) versus the pH and TA values for CW samples as a function of ageing time.
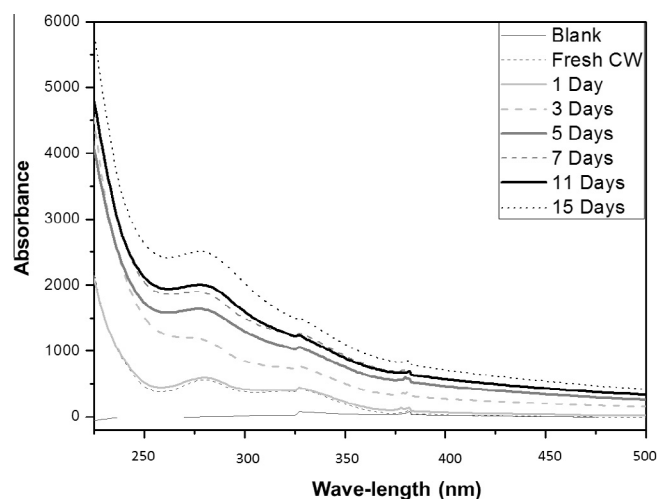

Fig. 5. UV–vis spectra of CW samples submitted to natural ageing conditions.

was easily confirmed when we observed the isotopologue pattern (the relative intensity of the m/z M+35Cl ion was 1/3 of that of m/z M+37Cl). A clear and evident change in the chemical profile of CW was observed after 3 days of natural storage in the m/z 150–250 and 350–450 regions using negative ion mode ESI. Ten new species were identified. Initially, the relative intensity of the natural markers (m/z 215 and 377 ions–sugar molecules) decreased as a function of ageing time. Consequently, several acid species were detected as gluconic acid (m/z 195); that is, the most abundant species.

Physicochemical analyses of the pH and TA showed changes after 3 days of storage (from 5 to 4.3 and from 0.08 to 0.36 g of citric acid per 100 mL of sample), making it unsuitable for consumption. TSS versus ageing time showed variations after 7 days of opening and storage of CW (from 5 to 4.3 °Bx) as a consequence of the reduction in sugar concentration. When the TSS/AT ratio was then plotted, a maximum plateau was reached (being indicative of high quality), and it remained constant until 3 days of ageing. In general, the ESI(−)-FT-ICR MS data were in good agreement with the physicochemical properties of CW, whereby the chemical species that were present, such as fructose/glucose, sucrose, and gluconic acid, could be used as natural markers to predict the quality of the fruits.

In the future, the ESI-FT-ICR technique will be applied to classify and evaluate the quality of CW and other fruits (mango, pineapple, etc.) as a function of the maturation stage, variation between species, soil components, and type of industrial conservation.

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