Food Research International 51 (2013) 467–473

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (*Mauritia flexuosa* L. f.) by UPLC–ESI-MS/MS

Hector H.F. Koolen [a,*], Felipe M.A. da Silva [b], Fábio C. Gozzo [a], Antonia Q.L. de Souza [c], Afonso D.L. de Souza [b]

[a] Intituto de Química, Universidade Estadual de Campinas, 13084-971, Campinas, SP, Brazil
[b] Departamento de Química, Universidade Federal do Amazonas, 69077-000, Manaus, AM, Brazil
[c] Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, 69-065-001, Manaus, Amazonas, Brazil

ARTICLE INFO

Article history:
Received 19 September 2012
Accepted 16 January 2013

Keywords:
Antioxidant activity
Buriti
Mauritia flexuosa
Phenolic compounds
UPLC–ESI-MS/MS

ABSTRACT

Mauritia flexuosa L. f. is a native palm tree from the South American Amazon rainforest commercialized in food, cosmetics and medicine. This paper reported the antioxidant, antimicrobial activities and characterization of phenolics from buriti leafs, trunk and fruit extracts. The total phenolics of the buriti extracts ranged from 378.07 ± 3.12 to 86.89 ± 3.15 mg GAEq/100 g and flavonoid content ranged from 567.16 ± 1.15 to 246.84 ± 1.11 mg QUEREq/100 g. The antioxidant activity assays with EC_{50} of 12.28 ± 0.022 to 19.58 ± 0.064 mg/mL revealed the antioxidant capacity of *M. flexuosa* extracts. Results of the antimicrobial tests against some pathogenic bacteria revealed its strong ability to inhibit the growth of pathogen with low MIC values, where the best results were found with the leaf extract against the pathogen *Pseudomonas aeruginosa* (MIC 50 μg/mL). The characterization of the phenolic compounds using Ultra Performance Liquid Chromatography coupled to Electrospray Ionization Tandem Mass Spectrometry (UPLC–ESI-MS/MS) was based on CID experiments of the observed precursors, thus 13 compounds were tentatively identified ((+)-catechin, caffeic acid hexoside, chlorogenic acid, quercetin, narigenin, myricetin, vitexin, scoparin, rutin, cyanidin-3-rutinoside, cyanidin-3-glucoside, (−)-epicatechin and kaempferol). These results indicate that antioxidant and antimicrobial activities in buriti phenolic extracts are quite potent and implicate the

1. Introduction

Natural antioxidants protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (Zheng & Wang, 2001). The biological properties of these natural antioxidants have been largely attributed to their high levels of various phenolic compounds, such as phenolic acids and flavonoids (Garzon, Narváez, Riedl, & Schwartz, 2010). The regular consumption of fruits has been associated with lower incidence and lower mortality rates of cancer in humans (Dragsted, Strube, & Larsen, 1993). The prevention of cardiovascular diseases, inflammations and aging-related disorders can also be correlated with intake of fruits which are rich in phenolic compounds and vitamins (Huang, Ou, & Prior, 2005). The Amazon region has suitable climatic conditions for a large number of underexploited native and exotic palm trees with interest to the agricultural industry, which can offer a future income source for local people. Buriti (*Mauritia flexuosa* L. f.), belonging to the Arecaceae Family and the genus *Mauritia*, is a palm widely distributed in the Amazon Rain Forest of Brazil (Delgado, Couturier, & Mejia, 2007). This dioecious tropical palm has high ecological, cultural and economic value. Destructive harvesting practices to obtain the fruits undermine the palm's potential in rural and regional economies (Cunha et al., 2012). Swamps which are dominated by *M. flexuosa*, are popularly known as buritizais in Brazil, occurring throughout the Amazon region and making its resources accessible to the harvesters in large quantities (Koolen et al., 2012). This species has high economic potential, due mainly to its fruits that have valuable oil for the cosmetic industries (França, Reber, Meireles, Machado, & Brunner, 1999). The oil extracted from the buriti fruits is popularly used against burns and as a potent vermifuge (Koolen et al., 2012). Those activities are attributed principally to the carotenoids and tocopherols, principal compounds in the oil (França et al., 1999). It is of interest of the general public, medical experts and food science researchers to know the antioxidant capacity and the major phenolic compounds present in the consumed foods. Based on this interest, the objectives of this study were to evaluate the antioxidant capacity and phenolic content of buriti fruits, once previous works with this fruit, just revealed the terpenoid content of the oil and its biological activities (Vásquez et al., 2010) beyond the ascorbic acid content of the fruits (Calderón, Calderón-Jaimes, Hernández, & Villanova, 2011). In addition, the antimicrobial activity of the phenolic extracts was evaluated against some pathogenic bacteria. Beyond the biological tests, the major phenolic compounds in the tested extracts were tentatively identified by Ultra Performance Liquid Chromatography coupled to Electrospray Ionization

* Corresponding author. Tel.: +55 19 8311 1441.
E-mail address: Hectorkoolen@gmail.com (H.H.F. Koolen).

0963-9969
http://dx.doi.org/10.1016/j.foodres.2013.01.039

Tandem Mass Spectrometry (UPLC–MS/MS) analysis for the first time for the fruits of this species and compared with the phenolic content of the leaves and trunk.

2. Materials and methods

2.1. Chemicals

The chemicals used in this study were of analytical or chromatographic grade. The aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the gallic acid were purchased from Sigma-Aldrich (Steinheim, Germany). The Folin–Ciocalteu reagent (2,4,6-tris(2-pyridyl)-*s*-triazine) (TPTZ), the quercetin and the rezazurin dye were obtained from Fluka-Chemie (Buchs, Switzerland). The salts sodium carbonate (Na_2CO_3), sodium acetate ($C_2H_3NaO_2$) and ferric chloride ($FeCl_3$) were purchased from Merck (Darmstadt, Germany). The Mueller Hinton Broth culture medium and the agarose were obtained from Becton-Dickinson Biosciences (Franklin Lakes, USA). The methanol (MeOH), acetonitrile (MeCN), hexane, dichloromethane (CH_2Cl_2) and ammonium hydroxide (NH_4OH) were obtained from Tedia (Fairfield, USA). The water used in all of the analyses was ultrapure produced by a Milli-Q system, Millipore (Bedford, USA) (R = 18 MΩ cm).

2.2. Plant material and sample preparation

The leaves, trunk and green fruits of three different buriti specimens (*M. flexuosa*) were collected in the 108 km of the AM-010 highway in the Amazonas State, Brazil, during June of 2010. The green fruits were washed, and the nut was separated from the rest of the fruit and then discarded. The remaining parts (bark and pulp) together with the leaves and the trunk (200 g each) were extracted one time with a hexane: CH_2Cl_2 (1:1) mixture in due to remove the apolar constituents (fatty acids, steroids, terpenoids and carotenoids). The whole remaining fruit material, trunk and leaves were dried and extracted the same way with methanol:water 7:3 (3_B250 mL), for each part to improve the yield of the extraction, the obtained extracts were concentrated under reduced pressure yielding the extracts: leaf extracts (LE, 2.7 g), trunk extract (TE, 3.3 g) and fruit extract (FE, 1.8 g). For the ultra pressure liquid chromatography coupled to tandem mass spectrometry analyses (UPLC–MS/MS), the extracts had 0.5 mg dissolved in H_2O:MeCN (1:1) and the pH was adjusted to 6.00 with 30% NH_4OH.

2.3. Determination of total phenolics (TPs)

The TP content of the aerial parts of *M. flexuosa* was determined using the Folin–Ciocalteu reagent (Singleton & Rossl, 1965). Each of the crude extracts (1 g) was dissolved in pure water (25 mL). Aliquots (250 μL) were mixed with 1 mL of the Folin–Ciocalteu reagent and 1 mL of a 10% (w/v) Na_2CO_3 solution. The samples were then incubated at 30 °C for 1.5 h and the absorbance of each sample was measured at 765 nm. The TP content was calculated from a calibration curve, using gallic acid as standard (1.25 to 7.5 μg/mL).

2.4. Determination of total flavonoids (TFs)

The TF content was measured by the $AlCl_3$ method (Lamaison & Carnet, 1990). The extracts (0.5 g) were dissolved in pure water (25 mL). Aliquots (1.5 mL) of the solutions were added to equal volumes of a solution 6% (w/v) $AlCl_3 \cdot H_2O$. The mixture was vigorously shaken, and absorbance was read at 367.5 nm after 10 min of incubation. Flavonoid contents were expressed in mg quercetin equivalent/g dry weight.

2.5. DPPH free radical scavenging assay

The free radical scavenging activity of the crude extracts was evaluated by measuring the decrease of the absorbance of an ethanolic DPPH solution at 517 nm in the presence of the extract according to a previous methodology (Mensor et al., 2001). The crude extract (4 mg) was dissolved in 2 mL of ethanol, mixed with 1 mL of a 0.1 mM solution of DPPH in ethanol. A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. The reaction mixture was incubated for 30 min at 37 °C, and the absorbance was then measured at 517 nm. Results were expressed as the amount of the extract that caused 50% disappearance (EC_{50}):

$$\text{Percent of disappearance} = [(A_{control} - A_E)/A_{control}] \times 100\%,$$

where, $A_{control}$ is the absorbance of control sample and A_{sample} is the absorbance of sample with the crude extract.

2.6. Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was done according to a previous methodology (Li, Gao, Huang, Zhang, & Guo, 2011). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL CH_3COOH), pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5-mL $FeCl_3 \cdot 6H_2O$ solution. The crude extract (2 mg) in 1 mL distilled water was allowed to react with 2.0 mL of the FRAP solution for 10 min at 37 °C, then the absorbance was measured at 593 nm. The standard curve was linear between 100 and 1000 μmol of Fe(II)/g. The final results were expressed as the concentration of $FeSO_4$ in 1 g of sample (μM/g).

2.7. Antimicrobial assay

The minimal inhibitory concentrations (MICs) were determined by microbroth dilution assays as recommended by the Subcommittee on Antifungal Susceptibility Testing of the US National Committee for Clinical Laboratory Standards (NCCLS) using a previous methodology (Salvador et al., 2002). The assays were performed on 96 well plates with 100 μL of Mueller Hinton Broth (MHB), 100 μL of extract solutions and 5 μL of test bacterial suspensions at 1.0×10^7 CFU mL^{-1}. The extracts assayed were dissolved in dimethylsulfoxide (DMSO) at initial concentration of 500 μg/mL until 3.125 μg/mL, and the incubation was made at 37 °C for 24 h. The microorganisms tested were *Escherichia coli* (ATCC 87064), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus luteus* (ATCC 4698) and *Bacillus cereus* (ATCC 14579). The bioactivities were recorded as blue coloration in the wells after the use of resazurin dye. The bacteriostatic or bactericidal effects of the extracts assayed were observed by inoculation of the well materials on Mueller Hinton Agar plates after the tests. For positive controls the antibiotic norflaxacin (4 μg/mL) and tetracycline (4 μg/mL) were used for all the tested strains. As for negative control the dimethyl sulfoxide (DMSO) was used during the tests. Characterization of phenolic compounds by UPLC–MS/MS.

2.8. UPLC-ESI-MS/MS analyses

Ultraperformance liquid chromatography (UPLC) analyses were performed using a Waters Acquity ultraperformance liquid chromatography system (Milford, USA), equipped with a binary pump system (Waters). The UPLC analyses were performed using an Acquity UPLC BEH column (50 mm × 2.1 mm i.d., 1.7 μm particle size) (Waters) with a binary mobile phase. Solvent A was water and B was acetonitrile. Two chromatography methods were developed to characterize the phenolic profile in the buriti samples: the first one, to analyze anthocyanins and flavonoid glucosides (System 1), and the other one to analyze the flavonoid aglycones (System 2). The System 1, the gradient elution at 30 °C was as follows: 0–12 min, 5–100% (v/v) B; 12–13 min, 100% B isocratic; and 13–14 min, 100–5% B. System 2 used the same column temperature: 0–5 min, 5–60% (v/v) B; 5–7 min, 60% B isocratic; 7–12 min, 60–100% B;

12–13 min, 100% B isocratic; and 13–14 min, 100–5% B. In both analytical methods, the flow rate was 0.3 mL/min and the sample volume injected was 5 μL. The UPLC system was coupled to the mass spectrometer (MS): Quattro Micro API triple quadrupole (Waters, Manchester, U.K.) using a Z-spray electrospray ionization (ESI) source. The MS was operated in negative mode to analyze the phenolic compounds, both analyses were done in a single run. The data were acquired in scan mode using a m/z range of 300 to 650 in System 1 and 200 to 450 in System 2. The ionization source working conditions were as follows: capillary voltage, 3 kV; source temperature, 150 °C; cone gas flow rate, 80 L/h; desolvation gas flow rate, 800 L/h; and desolvation temperature, 350 °C. Nitrogen (>99% purity) and argon (99% purity) were used as nebulizing and collision (product ion scan, MS/MS) gases, respectively. Data acquisition was carried out with MassLynx v 4.1 software.

2.9. Statistical analysis

All analyses were run in triplicate and the results were expressed as mean ± standard deviation (SD). Differences between means were first analyzed using the ANOVA test and then *post-hoc* Tukey test ($p<0.05$).

3. Results and discussion

3.1. Total phenol and total flavonoid contents in buriti

Phenolic compounds are considered the most antioxidant active metabolites from plants (Bors, Michel, & Stettmaier, 2001). These types of compounds have the ability to donate hydrogen or electrons beyond their capacity to form stable radical intermediates. The TP contents in different parts of *M. flexuosa* pointed this palm tree as a rich source of polyphenolic compounds. The FE extract presented the highest amount of TPs (378.07 ± 3.12 mg GAEq/100 g), followed by LE (102.54 ± 0.12 mg GAEq/100 g), whereas the TE presented the lowest amount of TPs (86.89 ± 3.15 mg GAEq/100 g). The TFs ranged from 567.16 ± 1.15 to 246.84 ± 1.11, as milligrams quercetin/100 g dried weight. Again the highest TF content was found in the fruits, followed by the leaves (398.77 ± 0.3 mg QUEREq/100 g) and the lowest amount was found in the trunk (Table 1). The TP and TF content of some other typical Amazonian palm trees previously reported had higher values: tucumã (*Astrocaryum acualeatum* G. Mey.) (Barreto, Benassi, & Mercadante, 2009), bacaba (*Oenocarpus bacaba* Mart.) (Abadio-Finco et al., 2012) and açaí (*Euterpe oleraceae* Mart.) (Kang et al., 2012) (433.2 ± 10.4 CTEq mg/100 g FW, 1759.27 ± 1.01 GAEq mg/100 g FW and 133.4 ± 11.2 GAEq mg/100 g FW respectively). The TP ratios of the extracts from *M. flexuosa* were lower than those from other Amazonian typical palm fruits due to the presence of residual non-phenolic reducing compounds such as carotenoids that were not totally removed from the extractions. These compounds are present in huge amounts especially in the fruits (França et al., 1999). The TF results can also be affected by the presence of non-phenolic compounds reacting with the Folin–Ciocalteau reagent, such as nitrogen and thiol containing compounds (Everette et al., 2010).These compounds are in a lower concentration when compared with the phenolic compounds in palm fruits (Schauss et al., 2006). The variation of the levels of phenolic compounds observed in buriti when compared with other typical Amazonian palm fruits, can also be explained partly due to the differences in the growing conditions. Under field conditions, the phenolic composition of plant tissues varies considerably with seasonal, genetic and agronomic factors (Garzon et al., 2010). In addition, a large variability at various stages of maturation and for different growing conditions, such as temperature and rainfall, is known to affect the contents of phenolic compounds (Tomás-Barberan & Espín, 2001). The observed values of TPs and TFs did not correlated with the values obtained in the antioxidant capacity for the same samples. A possible cause is the observed chemical composition of the extracts, which differs on the types of flavonoids and anthocyanins found in the extracts. The structural features and the antioxidant capacity are intrinsically connected (Heim, Tagliaferro, & Bobilya, 2002). Based on this information, it's possible to justify the highest TPs and TFs for the FE extract, where a greater variety of phenolic compounds was found, being flavonoid and anthocyanin glycosides the major compounds. The greater complexity observed in the chemical composition can possibly create a negative synergism in the observed antioxidant results, once flavonoid aglycones are more potent antioxidants than their corresponding glycosides (Gao, Huang, Yang, & Xu, 1999).

3.2. Antioxidant activity of buriti phenolic extracts

In the FRAP assay, the results were expressed as the combined concentrations of all electron-donating reductants, which occur in the samples obtained from the different parts of *M. flexuosa*. As shown in Table 1, LE showed the best reducing ability (328.65 ± 12.56 μmol of Fe(II)/g), followed by TE (284.50 ± 16.98 μmol of Fe(II)/g), and FE (280.80 ± 37.99 μmol of Fe(II)/g). The FRAP assay measures the reducing ability of antioxidants that react with ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produces a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) (Benzie & Strain, 1996). In the DPPH tests the presence of antioxidant in the sample leads to the disappearance of DPPH radical chromogens which can be detected spectrophotometrically at 517 nm (Krings & Berger, 2001). The EC_{50} ranged from 12.28 ± 0.022 to 19.58 ± 0.064 mg/mL, the obtained results for the DPPH tests are in accordance with previous antioxidant capacities recorded for the buriti oil, but differences on the absolute values were expected due the different chemical compositions (Ferreira et al., 2011). The scavenging ability of the test samples was higher, consistent with the TF content, FE being the most active, followed by the leaves and the trunk. The results obtained for TP and TF displayed no linearity correlation with the antioxidant assay, which was previously observed for some ORAC assays (Ou, Huang, Hampsch-Woodili, & Flamanan, 2003). The differences in the antioxidant activity (Table 1) can be explained by the differences in phytochemical composition and concentration, primarily associated with flavonoids and anthocyanin content, and also for the presence of several glycosylated compounds in the buriti extracts. Previous studies with phenolics from palm have demonstrated antagonistic interactions between polyphenolics. The diversity of these phenols in buriti creates a complex matrix, as already cited in jussara (*Euterpe precatoria*) (Del-Pozo, Brenes & Talcott, 2004). The consideration of results from recent studies that have demonstrated the individual antioxidant activity of phenolic compounds in model systems beyond the interaction

Table 1
Total phenolics, total flavonoid and antioxidant capacity of the extracts of *M. flexuosa*.

sample	Total phenolics (TPs) (mg GAEq/100 g) $p<0.03$	Total flavonoids (TFs) (mg QUEREq/100 g) $p<0.04$	FRAP (mmol $FeSO_4 \cdot 7H_2O$ equiv/100 g) $p<0.03$	DPPH EC_{50}(mg/mL) $p<0.02$
LE	102.54 ± 0.12[a]	398.77 ± 0.3	328.65 ± 12.56	12.28 ± 0.022
TE	86.89 ± 3.15	246.84 ± 1.11	284.50 ± 16.98	13.23 ± 0.014
FE	378.07 ± 3.12	567.16 ± 1.15	280.80 ± 37.99	19.58 ± 0.064

Values are expressed as means ± SD (n = 3).
[a] Tukey test ($p<0.05$).

Table 2
Antimicrobial activity from the extracts of *M. flexuosa*.

sample	*S. aureus*	*P. aeruginosa*	*E. coli*	*M. luteus*	*B. cereus*
LE	50 μg/mL	50 μg/mL	n.a.[a]	200 μg/mL	n.a.
TE	100 μg/mL	n.a.	n.a.	n.a.	n.a.
FE	100 μg/mL	200 μg/mL	n.a.	200 μg/mL	n.a.

[a] Not active.

occurring between them, demonstrated that there's an increase or decrease in the antioxidant activity, that is, the synergistic or antagonistic effect of the compounds (Hidalgo, Sánchez-Morano, & Pascual-Teresa, 2010; Terpinc & Abramovic, 2010).

3.3. Antimicrobial activity of buriti phenolic extracts

Only a few works in the literature report the antimicrobial activity of edible palm fruits. The results of the antimicrobial activity recorded for the phenolic extracts showed weak and moderate activities against the tested bacteria (Table 2). The best results were found in the LE extracts with moderate activity against *S. aureus* and *P. aeruginosa* (50 μg/mL). The most consumed part of *M. flexuosa* (FE extract) presented only a weak antimicrobial activity against the same strains. The antimicrobial activity of buriti has been previously reported for the mature epicarps and mesocarps, which were extracted with ethanol and partionated with hexane and ethyl acetate, where there was observed high antimicrobial activity against *S. aureus* and *P. aeruginosa*, being the most active of the extracts resulting from the hexane portion (Silveira et al., 2005). The oil from the fruits of *M. flexuosa* in previous tests did not showed antimicrobial activity against some pathogenic bacteria (Ferreira et al., 2011). The obtained results can be related with the absence or short concentration of low polarity compounds due the mode of preparation of the tested extracts, which did not presented metabolites such as unsaturated fatty acids, which have proven to have antimicrobial activity (Nazif, 2002). Other possible cause can be assigned to the phenolic composition of the fruits. In a previous study other typical Brazilian palm fruit (*Cocos nucifera*) had its phenolic extract tested against several pathogenic bacteria strains using a different methodology (Chakraborty & Mitra, 2008), where the authors found a high antimicrobial activity for the mesocarps of coconut. The identification of the phenolics from *C. nucifera* is present in the tested extract point to the presence of different caffeoylshikimic acids as main compounds, which were not observed in *M. flexuosa* extracts.

3.4. Characterization of phenolic compounds by UPLC–MS/MS

Since polyphenols contain one or more hydroxyl and/or carboxylic acid groups, MS data were acquired in negative ionization mode. The phenolic compounds were tentatively identified on basis of the analysis from the deprotonated ions $[M-H]^-$ and the fragments released in MS/MS experiments. MS/MS spectra are very useful for identifying the aglycones of flavonoids, and the analysis of fragmentation patterns is

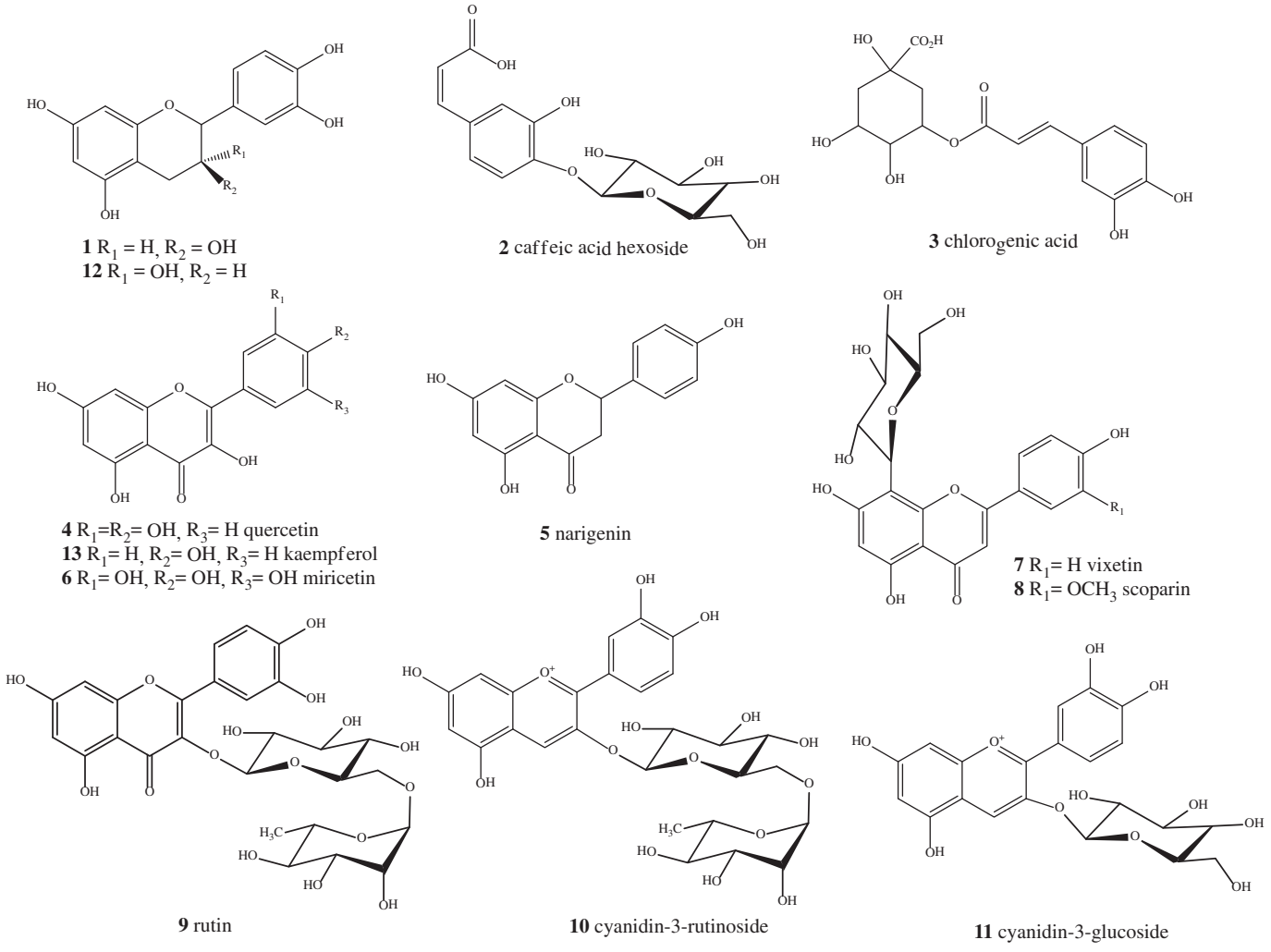

Fig. 1. Phenolic compounds identified in *Mauritia flexuosa*.

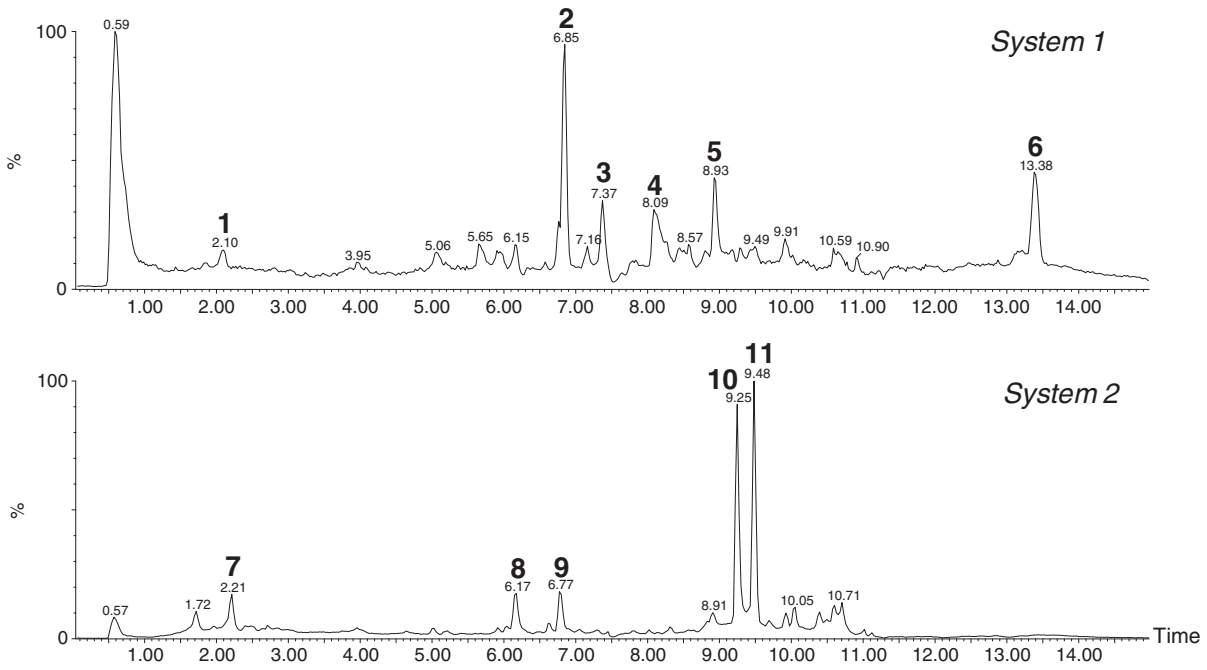

Fig. 2. Total ion currents (TICs) of the phenolic extract of the fruits from *Mauritia flexuosa*. System 1: Hydroxycinnamic acid derivatives and aglycone flavonoids. System 2: Flavonoid glucosides and anthocyanins.

highly diagnostic, allowing the elucidation of structures by comparison with the data in the literature (Fabre, Rustan, Hoffman, & Quentin-Leclercq, 2001). A total of 13 polyphenols (Fig. 1) distributed in the analyzed extracts have been analyzed in the present study. Fig. 2 shows the total ion current (TIC) chromatogram of the systems used in the analysis and the annotated major peaks observed in the fruits. The observed compounds, in which they detected part of the plant and the fragment ions were assigned in Table 3.

Two hydroxycinnamic acid derivates were identified in the extracts of different parts of *M. flexuosa*, the precursor ions at *m/z* 341 eluting at 6.85 min (FE, LE) and *m/z* 353 at 7.37 min (LE, TE and FE) were subjected to CID. The product ion spectra for the precursor at *m/z* 341 were displayed as the main fragment of the peak at *m/z* 179.0, result of a hexoside loss (162 Da). The mass of this fragment is coherent with the structure of the caffeic acid. Other typical fragments at *m/z* 161, 113 and 71 are unique diagnostic fragments of a caffeic acid moiety (Hossain, Rai, Brunton, Martin-Diana, & Barry-Ryan, 2010). These ions led to the identification of this ion as being a caffeic acid hexoside. The MS/MS of the precursor *m/z* 353 is consistent with the structure of the chlorogenic acid, the product ions at *m/z* 191 and 179 are related with the quinic acid and caffeic acid respectively revealing their structure.

Flavonoids are the main polyphenols present in the extracts of *M. flexuosa*. The precursor ions *m/z*, 317 (R_t 13.38 min), 285 (R_t 3.61 min) and 301 (R_t 8.09 min) presented the common fragments *m/z* 179 and 151 in the product ion spectra, as a result of the retrocyclization on the A–C ring moiety ($^{1,2}A^-$) and the consecutive loss of CO respectively (Fig. 3). Those are diagnostic ions of flavonol aglycones. Another fragments generated by the losses of CO and CO_2 were observed for the precursors. The fragmentation pattern led to the identification of these precursors as being myricetin (all extracts), kaempferol (LE, FE) and quercetin (FE) respectively, only quercetin was previously described in *M. flexuosa* (Koolen et al., 2012).

The CID spectrum of the precursor *m/z* 271 (R_t 8.93 min) (LE, TE) identified as the flavonoid narigenin, yielded a $[M–H–CO]^-$ base peak, which is related to flavanone typical fragmentation (Fabre et al., 2001). This small neutral loss is favored by the contracted C ring of the flavanones. The other fragments displayed for the precursor *m/z* 177 $[M–H\text{-ringB}]^-$ and 151 which resulted from a Retro Diels Alder fragmentation and formation of the $^{1,3}A^-$ ion (Ma, van den Huevel, & Claeys, 1999). The two peaks eluting at 1.83 (LE, TE) and 2.10 min (all extracts) displayed the same precursor mass *m/z* 289 and product ions (*m/z* 245, 203, 187 and 161). The comparison of the fragments

Table 3
Characterization of the individual phenolic compounds in buriti extracts using UPLC–ESI-MS/MS.

Peak	R_t[a]	Compound	$[M-H]^-$	Major fragments (*m/z*)	System	Detected in
1	2.10	(+)-Catechin	289	245, 203, 187, 161	1	FE, LE, TE
2	6.85	Caffeic acid hexoside	341	179, 161	1	FE, LE
3	7.37	Chlorogenic acid	353	191, 179, 173	1	FE, LE, TE
4	8.09	Quercetin	301	151, 179, 229, 257, 273	1	FE, LE, TE
5	8.93	Narigenin	271	227, 177, 151	1	LE, TE
6	13.38	Myricetin	317	151, 179, 245, 289	1	FE, LE, TE
7	2.21	Vitexin	431	341, 311, 283, 269	2	FE
8	6.17	Scoparin	445	341, 231	2	FE
9	6.77	Rutin	609	301	2	FE, LE, TE
10	9.25	Cyanidin-3-rutinoside	595	449, 287	2	FE
11	9.48	Cyanidin-3-glucoside	449	287	2	FE
12	1.83	(−)-Epicatechin	289	245, 203, 187, 161	1	LE,TE
13	3.61	Kaempferol	285	257, 229, 199, 151	1	TE

[a] Retention time.

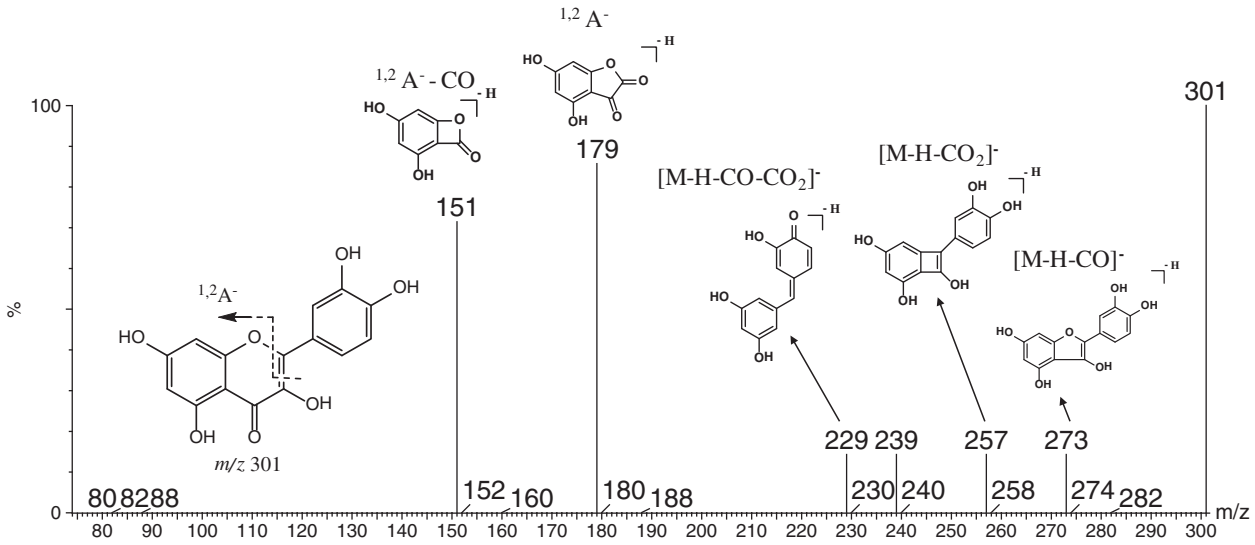

Fig. 3. ESI-MS/MS spectrum of product ion scan of quercetin (*m/z* 301).

observed with previous described data (Pacheco-Palencia, Duncan, & Talcott, 2009) allowed the identification of these peaks as being (+)-catechin and (−)-epicatechin.

The second system was used for the identification of possible flavonoid and anthocyanin glucosides. The peaks eluting at 2.21 min (all samples) and 8.17 min (FE) displayed precursor masses *m/z* 431 and 445, the product ion spectra displayed for both presented similar fragmentation pattern, which differs on an initial methyl loss (15 Da), the precursor masses and the product ions are in concordance with the flavonoid *C*-glucosides structures of vitexin and scoparin respectively (Waridel et al., 2001). The precursor ion *m/z* 609 eluting at 6.77 min (all extracts) when subjected to CID exhibits as product ion only the fragment *m/z* 301, this observed data are in concordance with the structure of the flavonoid rutin, which was previously identified in *M. flexuosa* (Koolen et al., 2012).

The peaks at 9.25 min and 9.48 min (FE) were assigned as being cyannidin-3-rutinoside and cyanidin-3-glucoside due the presence of the single fragment *m/z* 285 in their MS/MS spectra (Pacheco-Palencia et al., 2009).

4. Conclusions

The results of this study reveal that the selected species contain a considerable amount of phenolic compounds. The antioxidant assays reveal the antioxidant properties of *M. flexuosa*, but with lower values when compared with other typical Amazonian palm fruits. The antimicrobial tests demonstrated a moderate antimicrobial activity for the fruits, which was already expected by means of the chemical composition, showing the absence of fatty acids and phenolics derived from the shikimic acid. The analysis of the phenolic profile of *M. flexuosa* mainly pointed to the presence of glycosilated flavonoids and anthocyanins, responsible for the low antioxidant capacity of the fruits. The results of this work will contribute to the potential commercial application of buriti as an economic natural antioxidant in the Amazon region of Brazil, where it's largely consumed by the local population.

Acknowledgement

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa do Estado do Amazonas (FAPEAM) e Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP).

References

Abadio-Finco, F. D. B., Kammerer, D. R., Carle, R., Tseng, W. H., Boser, S., & Graeve, L. (2012). Antioxidant activity and characterization of phenolic compounds from bacaba (*Oenocarpus bacaba* Mart.) fruit by HPLC–DAD–MS^n. *Journal of Agricultural and Food Chemistry*, *60*, 7665–7673.

Barreto, G. P. M., Benassi, M. T., & Mercadante, A. Z. (2009). Bioactive compounds from several tropical fruits and correlation by multivariate analysis to free radical scavenger activity. *Journal of the Brazilian Chemical Society*, *20*, 1856–1861.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, *239*, 70–76.

Bors, W., Michel, C., & Stettmaier, K. (2001). Structure–activity relationships governing antioxidant capacities of plant polyphenols. *Methods in Enzymology*, *335*, 166–180.

Calderón, J. C., Calderón-Jaimes, L., Hernández, E. G., & Villanova, B. G. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International*, *44*, 2047–2053.

Chakraborty, M., & Mitra, A. (2008). The antioxidant and antimicrobial activity properties of the methanolic extract from *Cocos nucifera* mesocarp. *Food Chemistry*, *107*, 994–999.

Cunha, M. A. E., Neves, R. F., Souza, J. N. S., França, L. F., Araújo, M. E., Brunner, G., et al. (2012). Supercritical adsorption of buriti oil (*Mauritia flexuosa* Mart.) in ϒ-alumina: A methodology for the enriching of anti-oxidants. *Journal of Supercritical Fluids*, *66*, 181–191.

Delgado, C., Couturier, G., & Mejia, K. (2007). Mauritia flexuosa (Arecaceae: Calamoideae), an Amazonian palm with cultivation purposes in Peru. *Fruits*, *62*, 157–169.

Del-Pozo, D., Brenes, C. H., & Talcott, S. (2004). Phytochemical composition and pigment stability of açaí (Euterpe oleraceae Mart). *Journal of Agricultural and Food Chemistry*, *52*, 1539–1545.

Dragsted, L. O., Strube, M., & Larsen, J. C. (1993). Cancer-protetive factors in fruits and vegetables: Biochemical and biological background. *Pharmacology & Toxicology*, *72*, 116–135.

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin–Ciocalteau reagent. *Journal of Agricultural and Food Chemistry*, *58*, 8139–8144.

Fabre, N., Rustan, I., Hoffman, E., & Quentin-Leclercq, J. (2001). Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *Journal of the American Society for Mass Spectrometry*, *12*, 707–715.

Ferreira, B. S., Almeida, C. G., Faza, L. P., Almeida, A., Diniz, C. G., Silva, V. L., et al. (2011). Comparative properties of Amazonian oils obtained different extraction methods. *Molecules*, *16*, 5875–5885.

França, L. F., Reber, G., Meireles, M. A., Machado, N. T., & Brunner, G. (1999). Supercritical extraction of carotenoids and lipids from buriti (*Mauritia flexuosa*), a fruit from the Amazon region. *Journal of Supercritical Fluids*, *14*, 247–256.

Gao, Z., Huang, K., Yang, H., & Xu, H. (1999). Free redical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochimica et Biophysica Acta*, *472*, 643–650.

Garzon, G. A., Narváez, C. E., Riedl, K. M., & Schwartz, S. J. (2010). Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (*Vaccinium meridionale* Swartz) from Colombia. *Food Chemistry*, *122*, 980–986.

Heim, K., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism, and structure–activity relationship. *The Journal of Nutritional Biochemistry*, *13*, 572–584.

Hidalgo, M., Sánchez-Morano, C., & Pascual-Teresa, S. (2010). Flavonoid–flavonoid interaction and its effect on their antioxidante activity. *Food Chemistry*, *121*, 691–696.

Hossain, M. B., Rai, D. K., Brunton, N. P., Martin-Diana, A. B., & Barry-Ryan, C. (2010). Characterization of phenolic composition in Lamiaceae spices by LC–ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, *58*, 10576–10581.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*, 1841–1858.

Kang, J., Thakali, K. M., Xie, C., Kondo, M., Tong, Y., Ou, B., et al. (2012). Bioactivities of açaí (*Euterpe precatoria* Mart.) fruit pulp, superior antioxidant and anti-inflammatory properties to *Euterpe oleracea* Mart. *Food Chemistry*, *133*, 671–677.

Koolen, H. H. F., Soares, E. R., da Silva, F. M. A., de Souza, A. Q. L., Rodrigues-Filho, E., & de Souza, A. D. L. (2012). Triterpenes and flavonoids from the roots of *Mauritia flexuosa*. *Revista Brasileira de Farmacognosia*, *22*, 189–192.

Krings, U., & Berger, R. G. (2001). Antioxidant activity of some roasted foods. *Food Chemistry*, *72*, 223–229.

Lamaison, J. L. C., & Carnet, A. (1990). Teneurs en principaux flavonoids des fleurs de *Crataegeus monogyna* Jacq et de *Crataegeus laevigata* (Poiret D. C) en fonction de la vegetation. *Pharmaceutica Acta Helvetiae*, *65*, 315–320.

Li, X., Gao, W. Y., Huang, L. J., Zhang, J. Y., & Guo, X. H. (2011). Antioxidant and antiinflammation capacities of some pear cultivars. *Journal of Food Science*, *76*, 985–990.

Ma, Y. L., van den Huevel, H., & Claeys, M. (1999). Characterization of 3-methoxyflavones using fast-atom bombardment and collision-induced dissociation tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, *13*, 1932–1942.

Mensor, L. L., Fabio, S. M., Gildor, G. L., Alexander, S. R., Tereza, C. D., Cintia, S. C., et al. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, *15*, 127–130.

Nazif, N. M. (2002). Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity. *Food Chemistry*, *76*, 77–81.

Ou, B., Huang, D., Hampsch-Woodili, M., & Flamanan, J. A. (2003). When the east meets the west: the relationship between yin-yang and antioxidation–oxidation. *The FASEB Journal*, *17*, 127–129.

Pacheco-Palencia, L. A., Duncan, C. E., & Talcott, S. T. (2009). Phytochemical composition and thermal stability of two commercial acai species, *Euterpe oleracea* and *Euterpe precatoria*. *Food Chemistry*, *115*, 1199–1205.

Salvador, M. J., Ferreira, E. O., Pral, E. M. F., Alfieri, S. C., Albuquerque, S., Ito, I. Y., et al. (2002). Bioactivity of crude extracts and some constituents of *Blutaparon portulacoides* (Amaranthaceae). *Phytomedicine*, *9*, 566–571.

Schauss, A. G., Wu, X., Prior, R. L., Ou, B., Patel, D., Huang, D., et al. (2006). Phytochemical and nutrient composition of the freeze–dried Amazonian palm berry, *Euterpe oleraceae* Mart. (Açaí). *Journal of Agricultural and Food Chemistry*, *54*, 8598–8603.

Silveira, C. S., Pessanha, C. M., Lourenço, M. C. S., Neves-Junior, I., Menezes, F. S., & Kaplan, M. A. C. (2005). Atividade antimicrobiana dos frutos de *Syagrus oleracea* e *Mauritia vinífera*. *Revista Brasileira de Farmacognosia*, *15*, 143–148.

Singleton, V. L., & Rossl, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 114–158.

Terpinc, P., & Abramovic, H. (2010). A kinetic approach for evaluation of the antioxidant activity of selected phenolics acids. *Food Chemistry*, *121*, 366–371.

Tomás-Barberan, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, *81*, 853–876.

Vásquez, P., Freitas, L., Solís, V. T., Torres, R. P., & Mancini-Filho, J. (2010). Chemical characterization and oxidative stability of the oils from three morphotypes of *Mauritia flexuosa* L.f, from the Peruvian Amazon. *Grasas y Aceites*, *61*, 390–397.

Waridel, P., Wolfender, J. L., Ndjoko, K., Hobby, K. R., Major, H. I., & Hostettmann, K. (2001). Evaluation of quadrupole time-of-flight tandem mass spectrometry and ion-trap multiple-stage mass spectrometry for the differentiation of C-glycosidic flavonoid isomers. *Journal of Chromatography. A*, *926*, 29–41.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, *49*, 5165–5170.