A Chemometrics Approach for Nuclear Magnetic Resonance Data to Characterize the Partial Metabolome Banana Peels from Southern Brazil

1 CEB-Centre Biological Engineering, School of Engineering, University of Minho, Campus of Gualtar, Braga, Portugal, E-mail: saracardoso501@gmail.com
2 Plant Morphogenesis and Biochemistry Laboratory, Federal University of Santa Catarina, Florianopolis, SC, Brazil
3 Agricultural Research and Rural Extension Company of Santa Catarina, Criciúma, Brazil
4 CEB-Centre Biological Engineering, School of Engineering, University of Minho, Campus of Gualtar, Braga, Portugal

Abstract:
Banana peels are well recognized as a source of important bioactive compounds, such as phenolics, carotenoids, biogenic amines, among others. As such, they have recently started to be used for industrial purposes. However, its composition seems to be strongly affected by biotic or abiotic ecological factors. Thus, this study aimed to investigate banana peels chemical composition, not only to get insights on eventual metabolic changes caused by the seasons, in southern Brazil, but also to identify the most relevant metabolites for these processes. To achieve this, a Nuclear magnetic resonance (NMR)-based metabolic profiling strategy was adopted, followed by chemometrics analysis, using the specmine package for the R environment, and metabolite identification. The results showed that the metabolomic approach adopted allowed identifying a series of primary and secondary metabolites in the aqueous extracts investigated. Besides, over the seasons the metabolic profiles of the banana peels showed to contain biologically active compounds relevant to the skin wound healing process, indicating the biotechnological potential of that raw material.

Keywords: nuclear magnetic resonance, chemometrics, banana, metabolite identification

DOI: 10.1515/jib-2017-0053
Received: August 21, 2017; Revised: October 4, 2017; Accepted: November 3, 2017

1 Introduction

Musa is a genus, part of the Musaceae family, that includes dessert bananas and plantains. Bananas are one of the leading fruit crops as source of energy worldwide and mainly for people living in the humid tropical regions [1], [2]. In this scenario, Brazil is one of the top producers of bananas in the world [2].

Traditionally, bananas are known as source of bioactive compounds able to promote wound healing, mainly from burns, and to help overcome or prevent illnesses such as depression [3], [4].

Although biologically active compounds typically occur in small quantities in plant biomasses [5], bananas have been recognized as a source of important pharmacologically-active compounds.

These compounds include phenolics, such as gallic acid and derivatives [6], which are secondary metabolites needed for normal cell growth and development [7]. Indeed, phenolic compounds are bioactive compounds known for their health benefits [8] as they have antioxidant properties and important biological effects, such as antibacterial and antiviral, vasodilatory, and protection against ultra-violet radiation, among others [7], [8]. Phenolics are beneficial to human health, due to their antioxidant and chelating properties, and antimitagenic and antitumoral effects [1]. However, in excess, they can limit the bioavailability of proteins, in the gastrointestinal tract [7].

Carotenoids, such as β-carotene and xanthophylls [9], are accessory pigments in photosynthesis and have been claimed to cause immune-enhancement and reduction of the risk of developing degenerative diseases, for instance [10], [11], [12]. Besides, pro-vitamin A carotenoids such as α- and β-carotene and β-cryptoxanthin are relevant constituents of the diet in less favoured populations in certain countries in south hemisphere [13]. In bananas, carotenoids are usually found in higher concentrations in the peel than in the pulp [14].

Biogenic amines, such as dopamine (DOPA) and L-3, 4 dihydroxyphenylalanine (L-DOPA), play an important role as neurotransmitters in the hormonal regulation of the glycogen metabolism in mammals [15], [16].
Moreover, DOPA, present in both banana pulp and peel, can be used to prevent or treat the Parkinson’s neurodegenerative disease [1]. Other bioactive compounds found in bananas include anthocyanins (delphinidin and cyaniding [17]), catechins (gallocatechin and epigallocatechin [18]), and sterols and triterpenes, such as β-sitosterol, stigmasterol, campesterol, and 24-methylene cycloartenol [19].

Unlike the pulp, banana peels, the main residual biomass of the processing industry, are normally used for animal feeding, as organic fertilizers or simply discarded [20]. The latter can cause serious environmental problems, as this product is a rich source of nutrients, like nitrogen and phosphorus, that can lead to imbalances in the soil and aquatic environments where they are discarded [21]. However, banana peels, which represent about 30% of the fruit, have recently started to be used for industrial purposes. Depending on the technology employed, they can be used either as ingredients for products with therapeutic activity, as functional compounds in human nutrition, prevention and health care [1].

The use of banana peels for industrial purposes depends on its chemical composition, a trait strongly affected by climatic factors, orchard management practices, genotype, and harvest time, among others.

In this context, this study investigated the banana peels chemical composition over the seasons in Southern Brazil, aiming to gain insights regarding eventual metabolic changes occurring along the harvest times of that fruit. For that, it was adopted a typical NMR-based metabolic profiling strategy coupled to metabolite identification and chemometrics tools, including both univariate (analysis of variance) and multivariate (principal component analysis and clustering) statistical analysis.

## 2 Materials and Methods

### 2.1 Data Collection

#### 2.1.1 Chemicals

Ultra-pure water was obtained through a reverse-osmosis system (Permuter E-10, Curitiba, Brazil). The deuterated solvent D2O was purchased from TediaBrazil (Rio de Janeiro, Brazil) and 3-trimethylsilyl propionic-2, 2, 3, 3-d4 acid sodium salt (98 atom % D–TSP) and deuterium chloride solution (35 wt.% in D2O, 99 atom % D) were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

#### 2.1.2 Samples

Thirteen banana peels samples were collected from an agro-ecologically managed orchard, in Biguaçu County (27 29' 39" S; 48 39' 20" W, altitude 2 m), Santa Catarina State, southern Brazil. Three samples were harvested in the autumn (March, April, and May-2011), four in winter (June-2011, July-2010/2011 and August-2011), five in spring (September-2010/2011, October-2010/2011 and November-2010), and one in summer (February-2011). The producing region is characterized for well-marked seasons. The sampled biomass was collected from ripe fruits, showing a yellow colour throughout the peel. Fruits were washed in tap water and the peels immediately immersed into liquid N2 to guarantee the metabolic quenching. Further aqueous extracts (AE) of the banana peels were obtained as described in Pereira, 2014 [22] and lyophilized.

#### 2.1.3 1D-NMR Spectroscopy

Lyophilized AEs were added of 700 μL D2O, containing 0.024 g % of 3-trimethylsilyl propionic-2, 2, 3, 3-d4 acid sodium salt (98 atom % D–TSP) as internal standard, vortexed (3×), and centrifuged (4000 rpm/10 min), followed by recovering the supernatant (650 μL) and transferring it to 5 mm-NMR tubes. The pH of the samples was adjusted to 3.45 with a deuterium chloride solution (35 wt.% in D2O, 99 atom % D). The unidimensional NMR spectra (1H-NMR) were recorded in a Varian Inova 500 MHz NMR spectrometer and the chemical shifts (δ, ppm) were referenced to the TSP peak at δ (1H) 0.00 ppm. Data acquisition used a Dell workstation and the VNMRJ software, running on Windows 7 platform. Briefly, 1H-NMR spectra acquisition parameters were as follows: 300 K, no spinning, spectral window 5995.7 Hz, acquisition time 4 s, complex points 32,983, scans 32, steady state 4, receiver gain 10, relaxation delay 6 s, observe pulse 8.18 us at a power compression 59/0.98, mixing time 100 ms for saturation of water (δ = 4.87 ppm, Watergate pulse), and digital resolution ±0.08657 Hz.
2.2 Data Processing

The $^1$H-NMR spectra were processed using the ACD/NMR processor software (advanced chemistry development, release 12.0) consisting of zero filling, Fourier transforming the 32 K data points, and automatically phased (Ph0 and Ph1). The baseline was manually corrected and all spectra referenced to the internal standard (TSP, $^1$H – 0.00 ppm). The spectroscopy information of interest was exported as a .csv file containing a matrix with the chemical shifts ($^1$H ppm) and a peak intensity list. Typical resonance regions of the water and internal standard (TSP) signals removed from the dataset for further analysis. Further, each $^1$H-NMR spectrum was processed using a routine implemented in the R language [23] through the package specmine [24]. Peak alignment grouped proximal peaks together according to their position using a moving window of 0.03 ppm. Peaks of the same group were aligned to their median positions across all samples. Also, missing value imputation was done filling with a constant value of 0.0005, and data pre-processing further contemplated log transformation and auto-scaling.

2.3 Chemometrics Analysis

The metadata considered in this study were the seasons of collection. However, as it was only possible to obtain one sample for the summer period, only three seasons were considered for data analysis. The season variable was therefore assigned as spring for samples from September and October (years 2010 and 2011) and November-2010; as summer/autumn for the samples from February, March, April and May-2011; and, finally, as winter for the June-2011, July-2010/2011 and August-2011 samples.

The chemometrics analysis was executed using the R specmine package. The analysis started with one-way analysis of variance (ANOVA) to test the difference in means between the metadata groups for each one of the seasons. Then, multivariate statistical analysis was performed, starting with hierarchical clustering, using an Euclidean distance between samples and complete linkage clustering, to evaluate how close samples are inside each season and between seasons. The former parameter was chosen because it tends to find compact clusters of approximately equal diameters and does not force clusters together due to single elements being close to each other, like in single linkage clustering. After that, K-means clustering was performed, by separating the samples in three or four groups, to observe if samples would be well grouped according to their seasons, either by the three seasons considered or by the four actual seasons. Finally, the analysis was followed by principal components analysis (PCA).

2.4 Metabolite Identification

To perform the identification of the metabolites present in the samples, firstly a code developed by the authors in R for the clustering of peaks, according to their correlation, was used based on the methods proposed in [25]. Each obtained cluster is considered a potential metabolite, as it can be assumed that peaks coming from the same molecule, showing similar behaviour across all samples and, therefore, correlate strongly with each other.

After this each cluster was compared with each reference metabolite, using the Jaccard index score. The Jaccard index is used to compare the similarity between sets, as it is defined by the division of the size of intersection by the size of the union of the sets. A peak tolerance of 0.03 ppm was used, as in [25] it showed to be the best threshold value to use with the reference metabolites from the database used, mentioned below.

To construct the library of reference metabolites for this study, the XML spectra files from the Human Metabolome Database (HMDB) [26], version 3.6, were downloaded and parsed. Only the metabolites whose spectra files were acquired using the $^1$H-NMR technique, with a frequency of 500 MHz, were used as reference. For each cluster, the top five reference metabolites with the best score were obtained.

Additionally, metabolite identification was performed by visual inspection of the resonances both in unidimensional ($^1$H-NMR) and bidimensional ($^1$H/$^1$H, TOCSY and $^1$H/$^{13}$C, HSQC) NMR spectra.

2.5 Supplementary Material

The data used in the analysis, together with the reports generated using R Markdown are all given in supplementary material available in the URL: http://darwin.di.uminho.pt/pacbb2017/banana-nmr. This allows for the results to be understood in detail and fully reproducible.
3 Results and Discussion

3.1 Chemometrics Analysis

Starting our analysis of the results by observing the spectral profiles obtained for each sample collected, we could see that the samples have, approximately, the same peaks. When constructing the mean $^1$H-NMR spectra for each group considered (Figure 1), we observed that the peaks did not vary much between seasons. Despite this, the intensity of some peaks varied. Thus, although the banana samples collected seems to have the same metabolites, their concentrations vary from season to season.

**Figure 1:** $^1$H-NMR mean spectra plots for each season. Each plot was obtained from the ppm mean of the different samples for each season. (A) Spring season. (B) Summer/Autumn season. (C) Winter season.

Looking at the hierarchical clustering performed, shown in Figure 2, the samples were grouped in the considered seasons quite well. The samples from winter grouped very closely, except for the sample from July 2011, that was closer to the samples from May 2011 (summer/autumn) and September 2011 (spring). Adding this, the other three winter samples seem to be closer to the samples from April 2011, from summer/autumn and October 2011, spring, than these samples are to their groups. Furthermore, the spring samples were also well grouped, apart from the already mentioned ones from September 2011 and October 2011. On the contrary, the summer/autumn group revealed to be the most difficult one to group, not just regarding the summer sample in comparison to the autumn samples, but also between the autumn samples analysed.

**Figure 2:** Dendrogram plot of the hierarchical clustering, with Euclidean distance between samples. Spring samples are in black, summer/autumn samples in red and winter samples in green.
Another clustering method performed was $K$-means clustering. Two executions of this method were performed, one grouping the samples into three groups and the other into four. The results from both these approaches are present in Table 1.

| $K$-means Clustering | $K=3$ | $K=4$ |
|----------------------|-------|-------|
| Clusters             |       |       |
| June 2011 | July 2010 | June 2011 | July 2010 |
| July 2011 | August 2011 | July 2011 | August 2011 |
| October 2011 | September 2011 | October 2011 | |
| May 2011 | September 2010 | May 2011 | September 2010 |
| February 2011 | March 2011 | February 2011 | March 2011 |
| April 2011 | September 2011 | April 2011 | September 2011 |
| October 2010 | November 2010 | October 2010 | November 2010 |

Table 1: $K$-means clusters for clustering into three groups ($K=3$) and into four groups ($K=4$).

Spring samples are in Bold, summer/autumn samples in Italic and winter samples in Bold/Italic.

When performing $K$-means clustering, all winter samples were grouped together, along with the sample from October 2011 (spring). This was an expected result, after analysing the previous results from the hierarchical clustering. The samples from May 2011 and September 2011 were also grouped together in one of the clusters. This similarity was also previously observed in the hierarchical clustering.

When performing $K$-means clustering to separate samples in three different groups, the remaining summer/autumn and spring samples were grouped together. This is a result that could be expected, as autumn and spring are two seasons that normally might show similar climate conditions in southern Brazil. Furthermore, the difference for the $K$-means clustering for four different groups lies on the separation of this last cluster into two clusters: February 2011 and March 2011 form a separate cluster from the other samples.

Some of these inconsistencies in separating the samples in the right groups may be due to abnormal climatic conditions from the months in question. In fact, according to CPTEC/INPE (Center for Weather Forecasting and Climate Research/National Institute of Space Research) (http://clima1.cptec.inpe.br/monitoramento/pt) in October 2011 were recorded abnormally lower precipitation, while the months of June and July showed abnormally higher precipitation, revealing values very similar, which are not typical. Therefore, this might have been one of the factors to why October 2011, a spring sample, was grouped closer to the winter samples in both clustering methods.

Looking at the results from ANOVA, few were the peaks that showed a low corrected $p$-value, i.e. few peaks showed to have intensity means significantly different across samples from different seasons. The $p$-values were corrected by using the false discovery rate (FDR) method. After this correction, three peaks showed a corrected $p$-value below 0.1, as it can be observed in Table 2.

| Peaks | FDR   | Tukey result             |
|-------|-------|--------------------------|
| 1.89  | 0.0809| Spring – Winter;         |
|       |       | Summer/Autumn-Winter;    |
| 4.01  | 0.0809| Spring-Winter;           |
|       |       | Summer/Autumn-Spring     |
| 4.05  | 0.0809| Spring-Winter;           |
|       |       | Summer/Autumn-Winter     |

Table 2: ANOVA results for the peaks with the best corrected $p$-values (FDR method).

The first column contains the considered peaks, the second one the respective corrected $p$-value, and the final column the result of the Tukey’s test, which consists on the pair of groups that were significantly different in terms of means for each peak.

Furthermore, all peaks have means significantly different between the pairs of groups spring and winter, and summer/autumn and winter. Only the peak at 1.89 ppm showed a mean significantly different between summer/autumn and spring. This seems to corroborate some observations taken from the previous analysis performed i.e. the winter and spring samples are the ones that are better distinguished, and the summer/autumn samples are not so well distinguished from the spring samples. The three peaks occur in the aliphatic (1.89 ppm) and anomeric (4.01 and 4.05 ppm) regions of the $^1$H-NMR spectrum.
On the other hand, the PCA analysis showed that the first three components generated led to a cumulative explanation of more than 50% of the data variability, as it can be seen in Figure 3. Only the first component is already able to explain more than 20% of the data variability.

![Figure 3: Screen plot of the PCA, showing the percentage of explained data variability for each principal component obtained. The blue line corresponds to the individual percentage and the red one to the cumulative percentage.](image1)

By further observing the pairs plot generated by these first three components (Figure 4), we can realize that PC1 allows the distinction of the winter and spring groups from summer/autumn, while PC2 leads to the distinction of the groups spring and summer/autumn from winter, and the PC3 discriminates the winter and summer/autumn seasons from spring.

![Figure 4: PCA pairs plot of the first three components. The variables in pink correspond to the spring group, the green ones to the summer/autumn group and the blue to the winter group.](image2)

### 3.2 Identified Metabolites and their Relevance

Metabolite identification was performed through a code developed in R language by the authors. According to their correlations, peaks were clustered and each set of peaks grouped has been considered a metabolite, as they come from the same molecule. Further, by comparing the clustered peaks with resonances of reference metabolites using the Jaccard index score, twenty-three metabolites have been detected as shown in Table 3.

**Table 3:** $^1$H and $^{13}$C chemical shifts and proton multiplicity for assigned compounds found in aqueous extracts of banana peels (cv. Prata Anã) produced in southern Brazil (Santa Catarina State).

| Metabolite | HMDB ID | Cluster peaks matched | Reference peaks matched $\delta_{ppm}$ | $^1$H [multiplicity, assignment – $J$ (H, H) in Hz]/$^{13}$C | Jaccard index |
|------------|---------|-----------------------|-----------------------------------------|-------------------------------------------------------------|--------------|
|            |         |                       | $^1$H                                   |                                                             |              |
None of the three peaks that revealed the lowest corrected \( p \)-value in the ANOVA analysis were found in a cluster. Because the code is developed so that only clusters with two or more related peaks are taken into consideration, this could mean that each one of these peaks belongs to metabolites whose spectra is only composed by one peak. In fact, after submitting each of these peaks to HMDB, the peak 1.89 matched, with a ppm tolerance of 0.03, with the peak of the acetic acid spectra (1.91 ppm). For the other two peaks, 4.05 and 4.01, the best match found was \( \beta \)-D-fructose (4.08 ppm, m, 3-CH; 4.05 ppm, m, 5-CH) as previously described [27]. However, the correlation between these two peaks was not considered enough, as the optimum correlation value calculated by the code (value that leads to the greater number of clusters) that two peaks have to have, at least, is 0.9, and their correlation was lower. Thus, those resonances have been tentatively assigned do that monosaccharide.

Taking into account a biochemical approach, the polar fraction of the banana peel metabolome recovered by the aqueous extracts revealed to be a chemically complex matrix, containing a series of distinct primary and secondary metabolites, e.g. carbohydrates and derivatives (sugar alcohols, e.g.), amino acids, nitrogenous bases, organic and phenolic acids, vitamin B6 (pyridoxine), and polyamines. Since the number of identified compounds allow one discussing several biochemical relevant issues to, e.g. nutritional, agronomic and phar-
metadata

macological areas, in line with previous studies of our research group [1], [22], focus will be put on the metabolites with claimed effects on the wound healing process of cutaneous tissues. Thus, banana peels collected over the seasons in southern Brazil contain important bioactive compounds of interest to human health. In fact, in the Brazilian folk medicine banana peel has a history of utility to promote wound healing when used topically [1], [3].

An important class of biologically active molecules in banana peels are the phenolic compounds. In the present study, NMR spectroscopy coupled to bioinformatic tools have been able to detect caffeic acid (3, 4-dihydroxycinnamic acid), 4-hydroxybenzoic acid and syringic acid (3, 5-dimethoxybenzoic acid) in the aqueous extracts investigated. The former is originated from the mevolanate-shikimate biosynthesis pathways as the benzoic acids are produced via the loss of a two-carbon moiety from phenylpropanoids. These phenolic compounds possess antioxidant activity and anti-inflammatory properties, as well as are cytotoxic toward certain tumor cell lines [28]. Caffeic acid, for instance, is recognized as a potent antioxidant due to the delocalization of an unpaired electron caused by the extended conjugated side chain. Besides, its o-dihydroxyl group forms a hydrogen bond, which creates a more stable configuration after breaking the O-H bond [29].

Another class of secondary metabolites found in bananas are the natural amines. At least fifteen bioactive amines and derivatives have been identified in bananas so far. Among these, the polyamines spermidine, spermine and putrescine are commonly found, being spermidine as herein shown the prevalent one, followed by its precursor putrescine [30]. These are secondary metabolites with well-known metabolic and physiological functions on the growth and development of plants, as well as presenting neuroactive, psychoactive and vasoregulatory effects in mammals. Importantly, polyamines are required in higher contents during periods of wound healing and post-surgery recovery [31] what could explain in certain extension the positive effects found by our research group in using standardized aqueous extracts of banana peels in the wound healing of mechanically damage-cutaneous tissue of isogenic Balb/C mice – Mus musculus [22]. In fact, arginine, a dibasic amino acid and its metabolites nitric oxide, proline and polyamines (spermidine, spermine and putrescine, e.g.) are quite important in wound healing, affecting all phases of the process. Through the arginase pathway, L-ornithine, proline and polyamines are produced, the latter directly stimulating cell proliferation [31] and favouring the regeneration of damaged tissues. Besides, more recently, it has been shown that expression levels of heparan sulphate in human skin and wounded tissues of mouse are well correlated with polyamine contents [32].

Finally, vitamin B6 is a long known to play a critical role in protein metabolism. All the three forms of that water-soluble vitamin, i.e. pyridoxine, pyridoxal and pyridoxamine occur in the human body, being stable under acidic conditions [33]. Regarding the effect of pyridoxine on the skin wound healing process, studies have shown in rats that upon deficiency a decrease in the content, synthesis and maturation of the skin collagen is found, with negative effects on the healing of excision and incision wounds [34].

4 Conclusion

The results revealed that the distinction of the banana peels metabolic composition according to the seasons is possible, mostly due to the peak intensity, i.e. the concentrations of the metabolites across the different seasons. This distinction is more noticeable in the winter and spring groups, as these were the ones that better grouped in both cluster analysis and showed more significant differences in means regarding the ANOVA analysis. Furthermore, the PCA analysis revealed that it is only necessary three principal components to explain more than 50 % of the data variability.

These results show that the different conditions of the seasons can, in fact, influence the composition of the banana peels. The NMR-based metabolomic analytical strategy herein shown seems to be capable of identifying the chemical heterogeneity of banana peels over the harvest seasons, allowing to obtain standardized extracts for further industrial applications. Finally, as regards to metabolite identification a series of primary and secondary metabolites has been detected, shading some light on the chemical complexity of the aqueous extracts investigated. As herein shown, one could easily determine through the metabolomic approach adopted the potential of banana peels as source of active compounds on the skin wound healing (e.g.) upon the effects of seasoning. However, it must be taken into consideration that the metabolite identification pipeline adopted does not consider the clusters only composed by one peak. Therefore, some valuable information may have been lost, as there are many metabolites whose spectra only has one peak. Further analysis on this topic should be done. Furthermore, as the peak intensities seem to be the main aspect that changes according to the seasons, further analysis on the calculation of concentrations of the identified metabolites should be taken into account as well.
Acknowledgements

To CNPq (National Counsel of Technological and Scientific Development) for financial support (Process n 407323/2013-9), to CAPES (Coordination for the Improvement of Higher Education Personnel), and EGA-RGI (Agricultural Research and Rural Extension Company of Santa Catarina). The research fellowship from CNPq on behalf of M. Maraschin is acknowledged. The work is partially funded by Project PropMine, funded by the agreement between Portuguese FCT and Brazilian CNPq. This study was also partially supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020 – Programa Operacional Regional do Norte.

Conflict of interest statement: Authors state no conflict of interest. All authors have read the journal’s publication ethics and publication malpractice statement available at the journal’s website and hereby confirm that they comply with all its parts applicable to the present scientific work.

References

[1] Pereira A, Maraschin M. Banana (Musa spp) from peel to pulp: ethnonpharmacology, source of bioactive compounds and its relevance for human health. J Ethnopharmacol 2015;160:149–63.
[2] Padam BS, Tin HS, Chye FY, Abdullah MI. Banana by-products: an under-utilized renewable food biomass with great potential. J Food Sci Technol 2014;51:3527–45.
[3] Balbach A. As frutas na medicina doméstica. 21st ed. São Paulo: MVP; 1945. 374 p.
[4] Kumar KP, Bhomik D, Duraliev S, Umadevi M. Traditional and medicinal uses of banana. J Pharmacogn Phytochem 2012;1:51–63.
[5] Kris-Etherton P, Hecker K, Bonanome A, Coval S, Binkoski A, Hilpert K, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002;13:71–88.
[6] Nguyen T, Ketsa S, van Doorn W. Relationship between browning and the activities of polyphenoloxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. Postharvest Biol Technol 2003;30:187–93.
[7] Szejdak A, Borowska E. Bioactive compounds and health-promoting properties of berry fruits: a review. Plant Foods Hum Nutr 2008;63:147–56.
[8] Cook NC, Samman S. Flavonoids – chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 1996;7:66–76.
[9] Subagio A, Morita N, Sawada M, Tanaka M, Hiyama T. Carotenoids and their fatty-acid esters in banana peel. Nutr Sci Vitaminol 1996;42:553–66.
[10] Kirsinsky N, Johnson E. Carotenoids actions and their relation to health and disease. Mol Aspects Med 2005;26:459–516.
[11] Tapiero H, Townsend DM, Tew KD. The role of carotenoids in the prevention of human pathologies. Biomed Pharmacother 2004;48:100–10.
[12] Voutilainen S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. Am J Clin Nutr 2006;83:1265–71.
[13] Erdman J, Bierer T, Gugger E. Absorption and transport of carotenoids. Ann N Y Acad Sci 1993;691:76–85.
[14] Rodriguez-Amaya DB. A guide to carotenoid analysis in food. Washington: ILST Press; 2001. 71 p.
[15] Kanazawa K, Sakakibara H. High content of dopamine, a strong antioxidant, in cavendish banana. J Agric Food Chem 2000;48:844–50.
[16] Seymour GB. Banana. In: Seymour GB, Taylor J, Tucker G, eds. Biochemistry of fruit ripening. London: Chapman and Hall, 1993:95–8.
[17] González-Montelongo R, Lobo MG, González M. Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. Food Chem 2010;119:1030–9.
[18] Perreira A. Determinación del perfil químico y de la actividad cicatrizante de extractos de casca de banana cultivar prata anã (Musa sp.) e o desenvolvimento de um curativo para pequenas lesões. PhD thesis. Universidade Federal de Santa Catarina, Florianópolis. 2014.
[19] R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria, 2008. ISBN 3-900051-07-0. url: http://www.R-project.org.
[20] Rojas J, Pantoja M, Pinto J. A R package for the integrated analysis of metabolomics and spectral data. Comput Methods Programs Biomed 2016;129:177–24.
[21] Benucci C, Debode C, Moing A. An efficient spectra processing method for metabolite identification from 1H-NMR metabolomics data. Anal Bioanal Chem 2013;405:5049–61.
[22] Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0-The Human Metabolome Database in 2013. Nucleic Acids Res 2013;41(Database issue):D801–7.
[23] Maraschin M, Somensi-Zeggio A, Oliveira SK, Kuhnen S, Tomazzoli MM, Raguzzoni JF, et al. Metabolic profiling and classification of propolis samples from Southern Brazil: an NMR-based platform coupled with machine learning. J Nat Prod 2016;79:13–23.
[28] Gaglione M, Malgieri G, Pacifico S, Severino V, D’Abrosca B, Russo L, et al. Synthesis and biological properties of caffeic acid-PNA dimers containing guanine. Molecules 2013;18:9147–62.
[29] Son S, Lewis BA. Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure – activity relationship. J Agric Food Chem 2002;50:468–72.
[30] Glória MB. Bioactive amines. In: Hui YH, Sherkat F, eds. Handbook of food science, technology and engineering. Boca Raton: Taylor and Francis, 2005. Volume 4.
[31] Gould A, Naidoo C, Candy GP. Arginine metabolism and wound healing. Wound Healing Southern Africa. 2008;1:48–50.
[32] Imamura M, Higashi K, Yamaguchi K, Asakura K, Furihata T, Terui Y, et al. Polyamines release the let-7b-mediated suppression of initiation codon recognition during the protein synthesis of EXT2. Sci Rep 2016;6:33549.
[33] Liepa GU, Ireton-Jones C, Basu H, Baxter CR. B vitamins and wound healing. In: Molnar JA, ed. Nutrition and wound healing. Boca Raton: CRC Press, 2007.
[34] Lakshmi AV, Diwan PV, Bamji MS. Wound healing in pyridoxine deficiency. Nutr Res 1988;8:1203–06.