Characterization of the Chemical Composition of Banana Peels from Southern Brazil Across the Seasons Using Nuclear Magnetic Resonance and Chemometrics

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Abstract. Banana peels are a source of important bioactive compounds, such as phenolics, carotenoids, biogenic amines, among others. For industrial usage of that by-product, a certain homogeneity of its chemical composition is claimed, a trait affected by the effect of (a)biotic ecological factors. In this sense, this study aimed to investigate the banana peels chemical composition, to get insights on eventual metabolic changes caused by the seasons, in southern Brazil. For this purpose, a Nuclear Magnetic Resonance (NMR)-based metabolic profiling strategy was adopted, followed by chemometrics analysis, using the *specmine* package for the R environment. The obtained results show that the different seasons can, in fact, influence the metabolic composition, namely the levels of metabolites extracted from the bananas peels. The analytical approach herein adopted, i.e., NMR-based metabolomics coupled to chemometrics analysis, seems to enable identifying the chemical heterogeneity of banana peels over the harvest seasons, allowing obtaining standardized extracts for further technological purposes of usage.

Keywords: Nuclear Magnetic Resonance · Chemometrics · Banana

1 Introduction

In a worldwide scenario, Brazil is traditionally known as an important banana producer. Banana peel represents about 30% of the fruit and is the main residual biomass (by-product) of the processing industry. Such a by-product has an environmental significance, since it is a rich source of nutrients (e.g. nitrogen and

phosphorus) which could lead to imbalances in soil and aquatic environments [1]. On the other hand, the use of banana peel for industrial purposes depends on its chemical composition, a trait strongly affected by, e.g., climatic factors, orchard manage practices, genotype, and harvest time.

Banana is well recognized as source of important bioactive compounds, such as phenolics (gallic acid and derivatives [2]), carotenoids (β -carotene and xanthophylls [3]), anthocyanins (delphinidin and cyanidin [4]), biogenic amines (DOPA and L-DOPA [5]), catechins (galocatechin and epigallocatechin [6]), and sterols and triterpenes (β -sitosterol, stigmasterol, campesterol, and 24-methylene cycloartanol [7]). Besides, for industrial purposes, large amounts of banana peels must be provided, with homogeneous chemical composition, guaranteeing a continuous furnishment of raw material of high quality.

Thus, this study investigated the banana peel's chemical composition over the seasons, aiming to gain insights regarding eventual metabolic changes occurring along the harvest times of that fruit in southern Brazil. For that, a typical NMR-based metabolic profiling strategy coupled to chemometrics tools has been adopted, where the data analysis workflow includes both univariate (analysis of variance) and multivariate (principal component analysis and hierarchical clustering) statistical analysis.

2 Materials and Methods

Chemicals: Ultra-pure water was obtained through a reverse-osmosis system (Permutation 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).

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 in the autumn (March, April, and May-2011), four in winter (June-2011, July-2010/2011, and August 2011WI), 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 color throughout the peel, dried at 45 °C until constant weight and crushed in a mortar and pestle, using liquid N₂. Further aqueous extracts (AEs) of the banana peels were obtained as described by Pereira, (2014) [8] and lyophilized.

1D-NMR spectroscopy - spectrum acquisition parameters: Lyophilized AEs were added of 700 μ L 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 (3x), and centrifuged (4000 rpm/10 min), followed by recovering the supernatant (650 μ L)

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 $\delta(^1\text{H})$ 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 32983, scans 32, steady state 4, receiver gain 10, relaxation delay 6 s, observe pulse 8.18 μ s at a power compression 59/0.98, mixing time 100 ms for saturation of water ($\delta = 4.87$ ppm, Watergate pulse), and digital resolution ± 0.08657 Hz.

NMR Data Processing: The ¹H-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, d1_H0.00 ppm). The spectroscopy information of interest was exported as a .csv file containing a matrix with the chemical shifts (¹H pmm) 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 ¹H-NMR spectrum was processed using a routine implemented in the R language through the package *specmine* [9]. 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 contemplated log transformation and auto-scaling.

Chemometrics: The metadata taken into account was, as previously stated, the seasons. However, as it was only possible to obtain one sample for the summer, only 3 seasons were considered for the purpose of data analysis. The seasons were assigned as follows: the samples from September 2010/2011, October 2010/2011, and November-2010 were considered spring; the February, March, April, and May-2011 samples were considered summer/autumn and, finally, the June-2011, July-2010/2011, and August 2011 samples were considered winter.

The analysis of the obtained data was performed using the *specmine* package, as above, for the R environment [10]. The pipeline used for the data analysis started with one-way analysis of variance (ANOVA), to test the difference in means between the metadata groups for each one of the variables.

Then, multivariate statistical analysis was performed, starting with hierarchical clustering, using an euclidean distance between samples, followed by Principal Components Analysis (PCA).

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

The spectral profiles obtained for each sample showed that the samples have, approximately, the same peaks along the different samples.

In Fig. 1, we show the mean ^1H -NMR spectra of the samples from each of the different seasons considered. The report in supplementary material has all the samples represented.

Fig. 1. ^1H -NMR mean spectra plots for each season, obtained from the mean of the different samples plots for each season: A - Spring season. B - Summer/autumn season. C - Winter season.

Although the peaks seem not to vary across the different samples and, therefore, the seasons, the intensity of the peaks seems to slightly vary from season to season. This could mean that, despite having the same metabolites across samples, the concentrations of such metabolites vary from season to season.

The result for the hierarchical clustering can be observed in Fig. 2. It was possible to group the samples according to the metadata quite well. The samples from the autumn group were very close, except the sample from July 2011, that was more close to the samples from May 2011 (summer/autumn) and September 2011 (spring).

On the other hand, the other three winter samples seem to be closer to the samples from April 2011 (summer/autumn) and October 2011 (spring). Furthermore, the spring samples were also fairly well grouped, with the exception of the already mentioned samples from April 2011 and October 2011. It is noteworthy

Fig. 2. Dendrogram plot of the result of the hierarchical clustering, with euclidean distance between samples. Spring samples are in black, Summer/Autumn samples in red and Winter samples in green.

that these differences inside each season may be due to months that were hotter or colder than what is usual. Finally, the summer/autumn samples were, in general, very close to the spring samples.

The best results regarding ANOVA, i.e., the peaks whose corrected p-values were below 0.1, are present in the Table 1. The p-values were corrected by using the False Discovery Rate (FDR) method.

Table 1. ANOVA results for the peaks with the best corrected p-values (FDR method), also showing the pair of samples groups that were significantly different in terms of means for each peak.

Peaks	FDR	Tukey result
1.89	0.08090303	Spring-Winter; Sum/Aut-Winter; Sum/Aut-Spring
4.01	0.08090303	Spring-Winter; Sum/Aut-Winter
4.05	0.08090303	Spring-Winter; Sum/Aut-Winter

It is possible to realize that there were few peaks with low p-values. As it could be somewhat expected by observing the dendrogram plot in Fig. 2, all the three peaks with low p-values had means significantly different between the groups spring and winter, and summer/autumn and winter, as they were the group of samples that were further grouped in the dendrogram. In only one peak, significant differences in the means were observed regarding the summer/autumn and spring groups, as it was quite expected, due to the fact that they seemed very close in the dendrogram plot. The identified peaks occurs in the aliphatic

(1.89 ppm) and anomeric (4.01 and 4.05 ppm) regions of the ^1H -NMR spectrum, but, as such, do not allow further metabolite identification.

Finally, the results regarding the PCA analysis, present in Figs. 3 and 4, showed that the first principal component is able to explain more than 20% of the data variability, thus allowing to distinguish the groups winter and spring from summer/autumn. The next two components are able to explain, each one, more than 10% of the data variability, leading to an accumulative explanation of more than 50% of the data variability. The second component seems to be able to distinguish the groups spring and summer/autumn from winter, and the third component the groups winter and summer/autumn from spring.

Fig. 3. PCA results.

Fig. 4. Screeplot of the PCA results.

4 Conclusions

All the results showed that it is possible to distinguish the banana's peel metabolic composition according to the seasons, mostly due to the peak intensity. This distinction is more noticeable in the winter and summer/autumn groups, as were the ones that were better grouped in the cluster analysis and showed more significant differences in means regarding the ANOVA analysis. Furthermore, the PCA analysis revealed that it is only necessary 3 principal components to explain more than 50% of the data variability. This shows that the different conditions of the seasons can influence the composition of the banana's peel, addressing the need of further studies as one aims at to explore the potential of banana peel as source of bioactive compounds of interest of health and cosmetics industries, for instance. 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 obtaining standardized extracts for further industrial applications.

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