EXTENDED ABSTRACT

Journal of the International Society of Antioxidants
Issue n°4, Vol. 3 - 2016
DOI: 10.18143/JISANH_v3i4_1274

How polyphenols can help on the authentication of berry-based natural and pharmaceutical extracts by liquid chromatography-mass spectrometry techniques

NÚÑEZ Oscar[1,2,3], HIDALGO-SERRANO Míriam[1], BARBOSA Sergio[1], SAURINA Javier[1,2], MOYANO Encarnación[1], HERNÁNDEZ-CASSOU Santiago[1,2] & PUIGNOU Lluis[1,2]

[1]Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. Martí i Franquès 1-11, E-08028 Barcelona, Spain.
[2] Research Institute in Food Nutrition and Food Safety, University of Barcelona, Recinte Torribera, Av. Prat de la Riba 171, Edifici de Recerca (Gaudí), E-08921 Santa Coloma de Gramenet, Barcelona, Spain.
[3]Serra Húnter Program, Generalitat de Catalunya, Barcelona, Spain.

Corresponding Author:
Oscar Núñez
Department of Chemical Engineering and Analytical Chemistry, University of Barcelona
Martí i Franquès 1-11, E-08028 Barcelona, Spain
oscar.nunez@ub.edu

Abstract

Society is very interested in the quality of food products. Although consumer preferences are often influenced by organoleptic and socioeconomic factors, people are more interested in the presence of some specific compounds, such as polyphenols, with health beneficial properties. Polyphenols are aromatic secondary metabolites ubiquitously spread through the plant kingdom, and they caught the attention of consumers because of their antioxidant properties, their great abundance in our diet, and their probable role in the prevention of various diseases. However, nowadays there is a great concern about the possibility of frauds regarding the classification and authentication of, for instance, fruit processed products and pharmaceuticals.
In this work, the determination of polyphenolic profiles by liquid chromatography-high resolution mass spectrometry (LC-HRMS) techniques was explored for the characterization and authentication of berry-based natural and pharmaceutical extracts. For that purpose, more than 100 samples including cranberry-, blueberry-, raspberry-, and grape-based extracts and cranberry-based pharmaceuticals were analyzed using UHPLC-HRMS techniques, and the polyphenolic profiles submitted to principal component analysis for sample classification and authentication. The score-plot results showed that polyphenolic data obtained by high resolution mass spectrometry was very helpful in the classification and authentication of the analyzed samples, and several biomarkers were identified.

List of abbreviations: Polyphenols, pharmaceuticals, berry extracts, high resolution mass spectrometry, principal components analysis.

Received: 3 August 2016; Accepted: 21 December 2016
©Copyright JISANH 2016 – All rights reserved.

INTRODUCTION

The American red cranberry (*Vaccinium macrocarpon*) is a small evergreen shrub from the Ericaceae family that grows in acid swamps in humid forests. They are composed mostly of water and are a rich source of vitamin C and dietary polyphenols, such as flavonols, anthocyanidins, organic acids and proanthocyanidins (PAC). PACs are flavan-3-ol polymeric structures that are classified according to the linkage between units. PACs linked through C4-C8 or C4-C6 bonds are known as B-type PACs. If these structures have an additional ether linkage between C2 and C5 or C7 they are known as A-type PACs (Lin *et al.*, 2014). As an example, Figure 1 shows the structure of a trimeric PAC with A-type and B-type linkages. Cranberries have attracted much attention due to their content in A-type PACs. These polyphenols have been found to block the adhesion of bacterial fimbriae (e.g. *Escherichia coli*) to the epithelium of the urinary tract, which prevents the infection of the mucosal surface (Guay, 2009; Hisano *et al.*, 2012). This fact has triggered the appearance in the market of several cranberry-based pharmaceutical products (e.g., syrups, capsules or sachets) which claim to have a given concentration of PACs. However, the fact that only A-type PACs have the necessary bioactivity to prevent these infections and that the colorimetric assays used to quantify these compounds, such as the 4-(dimethylamino)-cinnamaldehyde (DMAC) method, are not capable of differentiating between PACs originating from cranberries (rich in A-type PACs) and the ones originating from other natural sources such as grape- or blueberry-based extracts, might allow producers to counterfeit their products with these more economical PAC sources while still providing acceptable specifications but without the appropriate polyphenols for having this desired bioactivity (Krueger, 2005). For this reason, it is important to develop analytical methodologies for the characterization of natural extracts to achieve unambiguous authentication regarding the type of fruit.

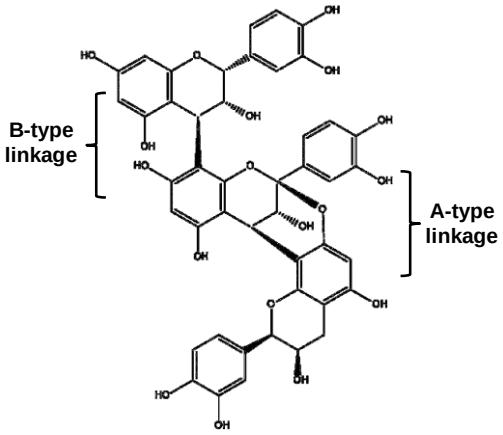

Figure 1. Structure of a PAC trimer with A- and B-type linkages.

MATERIALS AND METHODS

Chemicals and reagents

Unless otherwise stated, all reagents were of analytical grade. LC-MS grade water, acetonitrile and acetone, as well as formic acid (98-100%) were provided by Sigma-Aldrich (Steinheim, Germany), and hydrochloric acid (98%) was obtained from Merck (Seelze, Germany).

Samples and sample treatment

A total of 106 natural and pharmaceutical products were analyzed. Natural products from different brands were purchased from Barcelona (Spain) markets and include fruits, raisins and juices (33 cranberry-based, 29 grape-based, 12 blueberry-based and 10 raspberry-based). Pharmaceutical preparations (capsules, syrups and sachets) and raw extracts were provided by Deiters S.L. Company (Barcelona, Spain).

Prior to sample treatment, liquid samples were freeze-dried to achieve fully lyophilized products with a texture similar to that of natural extracts and commercial pharmaceutical preparations.

Sample treatment was carried out following a previously described method with some modifications (Parets *et al.*, 2016). Briefly, 0.1 g of sample was dispersed in 10 mL of acetone/water/hydrochloric acid (70:29.9:0.1 v/v/v) and sonicated for 30 min. After that, the mixture was centrifuged for 15 min at 3500 rpm, and the supernatant extract was separated from the solid and stored at -4 ^{o}C until analysis. Before injection, extracts were filtered through 0.45 μm nylon filters.

Received: 17 october 2016; Accepted: 21 December 2016
©Copyright JISANH 2016 – All rights reserved.

Apparatus and data analysis

Chromatographic separation was performed on an ultrahigh pressure liquid chromatography (UHPLC) system (Accela, Thermo Fisher Scientific, San José, CA, USA). An Ascentis Express C18 (150×2.1 mm, 2.7 µm) porous-shell column (Sigma-Aldrich) was employed for the separation. Gradient separation was achieved using solvent A (0.1% formic acid aqueous solution) and solvent B (0.1% formic acid in acetonitrile) as follows: 0−1 min, at 10% B; 1−20 min, linear gradient from 10 to 95% B; 20−23 min, at 95% B; 23−24 min, back to initial conditions at 10% B; and 24−30 min; at 10% B. Mobile phase flow rate was 300 µL/min, and the injection volume 10 µL.

The UHPLC system was coupled to a Q-Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a heated-electrospray (H-ESI) probe as the ionization source. Mass spectra were acquired in negative mode, at a resolution of 70,000 full width half maximum and with a scan range of 100-1,500 *m/z*.

High resolution mass spectrometry (HRMS) data was processed with Exact Finder v2.0 (Thermo Fisher Scientific) software. Data preprocessing and chemometrical analyses with principal component analysis (PCA) were carried out with *Solo*, provided by Eigenvector Research Inc. (Manson, WA, USA).

RESULTS AND DISCUSSION

Classification of analyzed samples was attempted by employing polyphenolic fingerprints acquired by UHPLC-H-ESI(-)-q-Orbitrap and PCA. The acquired UHPLC-HRMS data was processed by Exact Finder v2.0 software in order to search for specific polyphenols in the analyzed samples. A customized database of approximately 60 polyphenolic ions detectable in H-ESI(-) was applied to the data obtaining a response window where polyphenols were identified depending on whether they were confirmed (*m/z* and isotopic pattern matched), not confirmed (only *m/z* matched) or not found (*m/z* and isotopic pattern did not match) in the samples. After comparing chromatographic retention times and LC-MS/HRMS fragmentation, a matrix of peak areas was obtained with polyphenolic signals that were confirmed with a *peak score* higher than 0.65. PCA was then carried out on a matrix of size 106×75 after applying the chosen preprocessing of the data. The results obtained with different number of principal components (PC) were compared and the model built with 6 PC, which explained an 82.12% of the data variance with a root mean square error of cross-validation (RMSECV) of 0.7080 was chosen as the optimal one. The scores plot obtained under these conditions is shown in Figure 2.

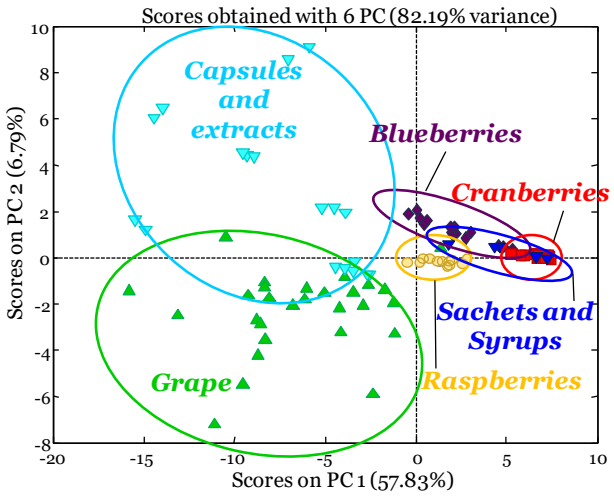

Figure 2. Scores plot (PC1 vs PC2) of the PCA obtained from the polyphenolic fingerprints.

An acceptable classification and discrimination between samples was achieved. Grape-based natural samples and cranberry-based capsules and extract pharmaceuticals appeared clearly separated at the left of the plot. Raspberry-based samples were well grouped at the center area, while the other samples tend to be at the center to the right area of the plot. In contrast to blueberry-based natural products which are more dispersed, cranberry-based natural products and cranberry-based sachets and syrups pharmaceutical preparations are more grouped and being distributed to the left of blueberry-based samples.

Cranberry-based natural products and pharmaceuticals did not form a clear cluster in the plot due to their differences in polyphenolic content coming from the manufacture processes of pharmaceutical preparations.

Some polyphenols were tentatively identified as possible future biomarkers to discriminate among samples. For instance, to distinguish cranberry from raspberry samples, rosmarinic acid and catechin seemed to be discriminant of the classes. Similar assumptions could be made for the differentiation of cranberry from blueberry samples (epicatechin and vanillic acid) or the differentiation of cranberry from grape samples (PAC B2, rosmarinic acid and gallic acid).

CONCLUSION

The results obtained in this work showed that UHPLC-HRMS polyphenolic profiles are a very suitable approach for the characterization and classification of berry-based

Received: 17 october 2016; Accepted: 21 December 2016
©Copyright JISANH 2016 – All rights reserved.

extracts according to their fruit of origin, and can be employed in the prevention of frauds in the production of cranberry-based pharmaceuticals.

ACKNOWLEDGEMENTS

This work has been funded by the Spanish Ministry of Economy and Competitiveness under the projects CTQ2014-65324 and CTQ2015-63968-C2-1-P, and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the projects 2014 SGR-377 and 2014 SGR-539.

REFERENCES

Guay DRP. 2009. Cranberry and urinary tract infections. Drugs 69, 775–807.

Hisano M, Bruschini H, Nicodemo AC, Srougi M. 2012. Cranberries and lower urinary tract infection prevention. Clinics 67, 661–667.

Krueger C. 2005. Authentication of Cranberry Ingredients. Natural Products Insider. Available at: http://naturalproductsinsider.com/articles/2015/03/authentication-of-cranberry-ingredients.aspx (Accessed on July 2016).

Lin L, Sun J, Chen P, Monagas MJ, Harnly JM. 2014. UHPLC-PDA-ESI/HRMS^n Profiling Method to Identify and Quantify Oligomeric Proanthocyanidins in Plant Products. Journal of Agricultural and Food Chemistry 62, 9387–9400.

Parets L, Alechaga É, Núñez O, Saurina J, Hernández-Cassou S, Puignou L. 2016. Ultrahigh pressure liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry for the determination of polyphenolic profiles in the characterization and classification of cranberry-based pharmaceutical preparations and natural extracts. Analytical Methods 8, 4363–4378.

Received: 17 october 2016; Accepted: 21 December 2016
©Copyright JISANH 2016 – All rights reserved.