Detection and Quantitation of Frauds in the Authentication of Cranberry-Based Extracts by UHPLC-HRMS (Orbitrap) Polyphenolic Profiling and Multivariate Calibration Methods

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Abstract

UHPLC-HRMS (Orbitrap) polyphenolic profiling was applied to the characterization, classification and authentication of cranberry-based natural and pharmaceutical products. 53 polyphenolic standards were characterized to build a user accurate mass database which was then proposed to obtain UHPLC-HRMS polyphenolic profiles by means of ExactFinder™ software. Principal component analysis results showed a good sample discrimination according to the fruit employed. Regarding cranberry-based pharmaceuticals, discrimination according to the presentation format (syrup, sachets, capsules, etc.) was also observed due to the enhancement of some polyphenols by purification and preconcentration procedures. Procyanidin A2 and homogentisic, sinapic, veratric, cryptochlorogenic and caffeic acids showed to be important polyphenols to achieve cranberry-based products discrimination against the other studied fruits. Partial least square regression allowed the determination of adulterant percentages in cranberry-fruit samples. Very satisfactory results, with adulteration quantification errors lower than 6.0% were obtained even at low adulteration levels.

Keywords: Polyphenols; Cranberry; Food characterization; Food Authentication; UHPLC; High resolution mass spectrometry; Orbitrap

INTRODUCTION

Food manufacturers and society are concerned about food product quality. Foodstuffs are complex products including, mainly, naturally occurring substances, but other compounds such as those migrating from packaging materials or those coming from technological and agrochemical processes can also be present. Typically, organoleptic and socioeconomic factors influence foodstuff consumer preferences. However, nowadays the presence of bioactive substances with healthy effects is gaining interest in the society. Unfortunately, fraudulent practices derived from food product adulterations by substitution, for instance, of the most valued components for others of lower commercial value and lower health beneficial properties are being employed to reduce food production costs.[1] For example, the addition of a co-fruit (a more economic and accessible fruit) to the final fruit-based processed foodstuffs such as juices is among the most common fraudulent practices that can be found in the fruit industry.[2] Fruit-based pharmaceutical preparations are also susceptible of fraudulent practices.

Cranberry (*Vaccinium macrocarpon*) and its derivatives have shown several health beneficial effects based on their ability to prevent urinary tract infections by hindering the adhesion of pathogenic bacteria to the urinary tract uroepithelial cells. This bioactivity is attributed to the presence of some specific flavan-3-ol polyphenols such as proanthocyanidins (PACs). These substances are classified into A-type and B-type PACs depending on the interflavan linkage between their monomeric units. When they are linked between the C6 or C8 positions of the lower monomeric unit and the C4 position of the upper monomeric unit they are considered B-type PACs. When an additional interflavan linkage through an ether-type bond between the C7 or C5 positions of the lower monomeric unit and the C2 position of the upper monomeric unit is present, the compounds are classified as A-type PACs.[3] However, only A-type PACs,

which accounts for more than 65% of the PAC content in cranberries, exhibit the bioactive activity to prevent urinary tract infections.[4-7] In contrast, B-type PACs, which are found in other fruits such as blueberry, raspberry and grapes, do not show this activity. Recently, some commercial pharmaceutical preparations supposedly produced only from cranberry extracts (and commercialized to prevent urinary tract infections) are adulterated with other less expensive fruit-based extracts (obtained from grapes or blueberries) poor in the desired bioactive polyphenols. This is because the overall contents of PACs are roughly assessed in pharmaceutical laboratories by a simple colorimetric analysis based on the reaction of PACs with 4-dimethylaminocinnamaldehyde (DMAC)[8,9] unable to differentiate among A- and B-type PACs. Thus, quality control of raw fruit extract materials (cranberry, blueberry, raspberry and grapes) as well as food-processed products require reliable, selective and effective methods for food authentication and for the prevention of frauds.

Nowadays, society is increasingly interested in polyphenols (aromatic secondary metabolites widely distributed into the plant kingdom) because of their great abundance in our diet, but mainly due to their role in the prevention of some diseases based on their antioxidant properties.[10-12] Furthermore, apart from their contribution to sensorial attributes such as the flavor and color properties of food products,[13,14] polyphenols have been recognized as relevant food descriptors. Polyphenolic content can be influenced by multiple parameters: environment climatic conditions, water availability sources, growing and cultivation techniques, the soil management practices, the degree of fruit maturation, etc. Thus, polyphenolic distribution and content can be used as analytical data to establish food authentication for correct product designations of origin (PDO) assignments and for the prevention of frauds. For instance, some fruit characteristic polyphenolic compounds have been successfully employed to detect frauds in nectars,

fruit juices and jams adulterated with cheaper fruits.[2,15,16] Thus, polyphenolic profiling and fingerprinting are very promising tools for the determination of food authenticity due to their taxonomic specificity in fruits.[16,17] For example, phlorizin and phloretin in the case of apples, arbutin in pears, naringenin derivatives in the case of citric fruits, and punicalagins (ellagic acid derivatives) for pomegranate, are specific polyphenols characteristic of the commented fruits.[2,15,18,20] Among polyphenols, anthocyanins are abundant in berries and grapes, and they have an strong influence in both flavor and color attributes. They have also been exploited by some authors as potential markers of grape varieties,[21-23] cherries,[24,25] blueberries[26] and other berries.[27] However, in some cases, the reported anthocyanin content on some berry fruits is inconsistent, fact that is unlikely ascribed only to geographical location and environment differences. Other factors such as the sample extraction methods employed and post-harvest actions including the storage conditions are more likely to explain these differences.[28,29]

The determination of polyphenolic compounds in foodstuff is complex not only because of the food matrix but also due to the diversity of polyphenols, with a great variability of chemical structures, that may be present. In addition, polyphenols have a wide range of polarities and sizes (simple phenolic acids, tannins, etc.), and they can be found in a wide range of concentration levels.[30] Thus, polyphenolic separation, determination and identification, as well as their sample extraction, are hindered by the chemical diversity within this family of compounds. The determination of polyphenols in fruit-based products is mainly addressed by liquid chromatography coupled to mass spectrometry or tandem mass spectrometry (LC-MS(/MS)) techniques. Electrospray as ionization source and triple quadrupole, ion-trap and linear ion-trap as MS analyzers are typically employed.[15,30-33] Recently, atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) have also been described for the mass

spectrometric ionization and determination of polyphenols.[34-36] Today, high resolution mass spectrometry (HRMS) techniques and the accurate mass measurements achieved with time-of-flight (TOF) and Orbitrap analyzers have also gained popularity in the characterization, identification and determination of polyphenols in foodstuffs.[30,37,38]

Lately, the use of polyphenolic compositional fingerprints and profiles as a source of information to achieve the classification of samples and their authentication in the prevention of frauds by means of chemometric methods is emerging.[17,30,39,40] The profiling approach employs the concentrations of targeted polyphenols as data, while in the fingerprinting approach data consists on instrumental signals such as intensity counts registered as a function of retention time and *m/z* values. The extraction of relevant information on descriptive and functional foodstuff characteristics to address the characterization and classification of products, and for authentication purposes, is achieved by further chemometric analysis of these data.[30]

This work aims at developing a UHPLC-HRMS (Orbitrap) method for the detection and quantitation of frauds in the authentication of fruit-based extracts by means of a targeted polyphenolic profiling and multivariate calibration. For that purpose, the 53-targeted polyphenols belonging to different families were fully characterized in terms of HRMS and product ion scan spectra with stepped normalized collision energies with accurate mass measurements, as well as retention time under reversed-phase separation conditions. An accurate mass database was built from such spectral and chromatographic data. Then, different classes of fruit-based (cranberry, blueberry, raspberry and grape) products including the raw fruit extracts, fruit juices and raisins, as well as commercially available cranberry-based pharmaceuticals including raw extracts, powder capsules, syrup, and sachets were analyzed after a simple sample extraction with acetone/water/hydrochloric acid (70:29.9:0.1 *v/v/v*). Data corresponding

to the 53-targeted polyphenolic compounds was employed as chemical descriptors to achieve the classification of the analyzed samples by principal components analysis (PCA). Partial least squared (PLS) regression was then applied to quantify fruit adulteration levels (grape, blueberry and raspberry) in cranberry samples.

MATERIALS AND METHODS

Reagents and solutions

Unless otherwise indicated, all the standards and chemicals used in this work were of analytical grade. Fifty-three polyphenolic standards belonging to different families (phenolic acids, benzoic acids, cinnamic acids, phenolic aldehydes, phenolic terpenes, flavones, flavanols, proanthocyanidins and stilbenes) were employed, and their chemical formula, CAS number and structure are given in Table 1. All the studied polyphenols were purchased from Sigma-Aldrich (Steinhein, Germany).

LC-MS grade water, methanol, acetonitrile, formic acid (98-100%) and acetone were also purchased from Sigma-Aldrich, and hydrochloric acid (98%) was from Merck (Seelze, Germany).

Stock standard solutions of all polyphenols (~1000 mg/L) were prepared in LC-MS grade methanol in amber-glass vials. Intermediate working solutions were prepared weekly from these stock standard solutions by appropriate dilution with LC-MS grade water. All stock solutions were stored at 4 ^{o}C for not more than 1 month.

Instrumentation

Chromatographic separation was carried out on an Accela UHPLC system (Thermo Fisher Scientific, San José, CA, USA), equipped with a quaternary pump, an autosampler and a column oven. A porous-shell Ascentis® Express C18 reversed-phase

column (150 × 2.1 mm, 2.7 µm partially porous particle size) provided by Supelco (Bellefonte, PA, USA) was used for the proposed method. Separation under gradient elution based on 0.1% formic acid aqueous solution (solvent A) and acetonitrile also containing 0.1% formic acid (solvent B) was as follows: 0-1 min, isocratic conditions at 10% B; 1-20 min, linear gradient from 10 to 95% B; 20-23 min, isocratic step at 95% B; 23-24 min back to initial conditions at 10% B; and from 24 to 30 min, isocratic conditions at 10%B to re-equilibrate the column. The mobile phase flow-rate was 300 µL/min, and the injection volume employed (in full loop mode) was 10 µL.

The UHPLC system was coupled to a Q-Exactive Orbitrap HRMS system (Thermo Fisher Scientific) equipped with a heated electrospray ionization source (HESI-II) operated in negative ionization mode. Nitrogen was used as a sheath gas, sweep gas, and auxiliary gas at flow-rates of 60, 0 and 10 a.u. (arbitrary units), respectively. HESI-II heater temperature at 350 ^{o}C and capillary voltage at -2.5 kV were applied. Instrument capillary temperature was set at 320 ^{o}C, and an S-Lens RF level of 50 V was used. Q-Exactive Orbitrap HRMS system was tuned and calibrated using commercially available Thermo Fisher calibration solution every three days. The HRMS instrument was operated in full MS scan mode with a *m/z* range from 100 to 1,500 at a mass resolution of 70,000 full width at half-maximum (FWHM) at *m/z* 200, with an automatic gain control (AGC) target (the number of ions to fill the C-Trap) of 1.0E6 with a maximum injection time (IT) of 200 ms. Full MS scan mode was followed by a data-dependent scan operated product ion scan mode and applying for the fragmentation stepped normalized collision energies (NCE) of 17.5, 35 and 52.5 eV. Product ion spectra with an isolation window of 0.5 *m/z* and a fixed first mass of *m/z* 50 were registered. At this stage, a mass resolution of 17,500 FWHM at *m/z* 200, with an

AGC target at 2.0e5 and a maximum IT of 200 ms were employed. Data dependent scan was triggered with an intensity threshold of 1.0E5.

Samples and sample treatment

106 samples including cranberry-based natural products (21 juices, 4 fruits and 8 raisins), grape-based natural products (17 juices, 4 fruits and 8 raisins), blueberry-based natural products (6 juices and 6 fruits), raspberry-based natural products (10 fruits), and cranberry-based pharmaceutical preparations presented in different formats (5 raw extracts, 11 capsules, 4 sachets and 2 syrups) were analyzed in this work. Natural fruit products were purchased from Barcelona markets. Juice products from different trademarks (Granini, El Corte Inglés, OceanSpray, Int-Salim and Lambda) were employed. Raisin samples were obtained from Barcelona markets and from several commercially available trademarks (Eroski and Hacendado). Cranberry-based pharmaceutical raw-extracts (Cysticran 40, several lots) were obtained from Deiters S.L. (Barcelona, Spain). Other cranberry-based pharmaceutical products (several lots) in different formats were obtained from the next sources: raw extracts Cysticran 40 from Naturex-DBS (Sagamore, MA, USA); sachets Cysticlean from Vita Green (Hong Kong, China) and sachets Urell from Pharmatoka (Rueil-Malmaison, France); capsules Cystop from Deiters, capsules Urell from Pharmatoka, capsules Cranberola Cis-control from Arkopharma (Madrid, Spain), capsules Urosens from Salvat (Barcelona, Spain) and capsules Monorelle from Zambon (Bresso, Italy); and syrup Urell from Pharmatoka.

An Ultra-Turrax machine from Ika (Staufen, Germany) was used to grind fruit and raisin samples. Raisin samples were mixed with water to help the cruising. Cranberry-based pharmaceutical syrups, fruits and raisins were freeze-dried to obtain completely lyophilized products (Telstar LyoQuest lyophilizer, Terrasa, Spain)

following the method described by Pardo-Mates *et al.*[3] Briefly, a 24 h gradient temperature ramp from -80 ºC to room temperature, followed by 6.5 h at 40 ºC, was employed for lyophilization.

Sample treatment was carried out following a previously described method with some modifications.[30,32,36,41,42] Briefly, 0.1 g of sample were extracted by sonication using 10 mL of an acetone:water:hydrochloric acid (70:29.9:0.1 v/v/v) solution, and the supernatant extracts obtained after centrifugation (3500 rpm, 15 min) were filtered (0.45 µm nylon filters, Whatman, Clifton, NJ, USA) and kept at -4 ºC until their analysis.

Besides, a quality control (QC) sample was prepared by mixing 50 µL of each sample extract. The QC was employed to evaluate the repeatability of the proposed method and the robustness of the chemometric results. All samples were analyzed randomly and QCs were introduced every ten samples.

Cranberry extracts (pure samples) were adulterated with different quantities of other fruits to perform authentication studies by PLS regression. Standard and unknown samples used in the PLS calibration and prediction sets were prepared using fruit extracts obtained as previously indicated. Pure extracts and cranberry-fruit adulterated extracts (from 2 to 50% adulteration levels) were employed.

Data analysis

HRMS raw data was processed by ExactFinder™ v2.0 software (Thermo Fisher Scientific) by applying a user target accurate mass database list comprising the 53 studied and characterized polyphenols. Parameters including chromatographic retention time, accurate mass errors, isotopic patterns and product ion spectra with steeped normalized collision energies were used for identification and confirmation purposes.

Stand Alone Chemometrics Software (SOLO) obtained from Eigenvector Research was employed for the calculations using PCA and PLS regression.[43] A theoretical background description of these chemometric procedures is described elsewhere.[44]

Data matrices to be treated by PCA consisted of the peak area values of the 53 studied polyphenolic compounds found in the analyzed samples. The dimension of the matrix was 106 samples x 53 analytes. Normalization pretreatment with respect to the overall polyphenolic concentration was applied to provide similar weighs to all the samples. The structure of the maps of samples and variables was investigated using the principal components (PCs) scatter plots of scores and loadings, respectively. The distribution of samples on the PCs (plot of scores) showed patterns that may be correlated to sample properties such as the type of fruit. In contrast, the distribution of variables on the PCs (plot of loadings) showed information regarding correlations and dependences of the studied polyphenols with the fruit products.

The percentage of fruit-extract adulterants (grape, blueberry or raspberry extracts) in the cranberry-based extracts was quantified by PLS. Samples available were distributed among training (calibration) and test (validation and prediction) sets (Table 1S in the supporting information). For both training and test steps, X-data matrices consisted of the polyphenol peak area signals of the corresponding samples and the Y-data matrices contained the adulteration fruit-extract percentages.

RESULTS AND DISCUSSION

HRMS characterization of targeted polyphenolic compounds

In the present work, a total of fifty-three polyphenolic standards belonging to different families (phenolic acids, benzoic acids, cinnamic acids, phenolic aldehydes,

phenolic terpenes, flavones, flavanols, proanthocyanidins and stilbenes) were analyzed by reversed-phase chromatography using a C18 fused-core UHPLC column under universal gradient elution conditions with water and acetonitrile (both 0.1% formic acid) as mobile phase components. Before sample analysis, HRMS characterization of targeted polyphenolic compounds was performed. For that purpose, targeted polyphenols were grouped in six standard solutions (preventing isobaric compounds) and analyzed with the proposed UHPLC-HRMS method (see experimental section) in negative ESI mode. Several parameters such as chromatographic retention times, HRMS spectra (at a resolution of 70,000 FWHM) and MS/HRMS product ion scan spectra (at a resolution of 17,500 FWHM) were established, and the data is summarized in Table 2. Although several coelutions were obtained within the analyzed polyphenols, these were clearly resolved by the high-resolution power of the Q-Exactive Orbitrap HRMS instrument. Regarding HRMS spectra, in general, all studied polyphenols provided as base peak the deprotonated molecule, $[M-H]^-$, which was then selected as the precursor ion for the MS/HRMS spectra (see as an example the HRMS spectrum of rutin in Figure 1a). As can be seen in Table 2, accurate mass measurements with errors bellow 1 ppm were obtained for almost all the analyzed compounds (49 of 53), and only 4 polyphenols (sinapic acid, epigallocatechin gallate, procyanidin C1, and protocatechuic aldehyde) showed slightly higher mass errors, although always below 5 ppm. It should be pointed out that generally no in-source fragmentation was observed during the HRMS experiments and for those cases where a slight in-source fragmentation was present the resulted signals were lower than 20% (relative abundance), hence they were not considered relevant for the intended study (see as an example the MS/HRMS spectrum of rutin in Figure 1b).

Because of the great variety of chemical structures among the studied polyphenols (see Table 1), MS/HRMS spectra were obtained by a data dependent acquisition mode based on product ion scan applying for the fragmentation stepped normalized collision energies (NCE) of 17.5, 35 and 52.5 eV. Thus, the product ion scan spectra were obtained as the average spectrum of the three collision energies. The observed fragment ions, assignments and accurate mass errors obtained are also summarized in Table 2. It should be mentioned that as the main objective of this work is to establish a fast targeted screening method to obtain discriminant polyphenolic profiles among the analyzed samples, optimal MS/HRMS conditions were not established for each compound, and data dependent scan mode was triggered only if the obtained signal for the targeted polyphenols was higher than 1.0E5. This would explain the fact that for some compounds no fragmentation was observed under the established acquisition conditions. As an example, Figure 1c shows the fragmentation pathway of rutin, one of the studied polyphenols, among others, that showed higher fragmentation under the applied conditions. Accurate mass measurements for all observed fragment ions with errors bellow 3.732 ppm were obtained.

Spectral data was employed to build a user accurate mass database of polyphenolic compounds for screening purposes with the ExactFinder^{TM} software.

UHPLC-HRMS polyphenolic profiling

UHPLC-HRMS polyphenolic profiles of fruit-based products and cranberry-based pharmaceuticals were studied in order to see if polyphenolic profiles resulted in proper chemical data to achieve sample classification and authentication. For that purpose, a total of 106 samples were processed with a simple sample extraction method and the obtained extracts were analyzed with a C18 reversed-phase UHPLC-HRMS

297 method using a fused-core column and a universal gradient elution profile (see
298 experimental section). Data was registered in HRMS full scan mode (*m/z* 100-1500) and
299 a data dependent scan mode based on product ion scan with stepped normalized
300 collision energies. As an example, Figure 2 shows the total ion chromatogram (TIC)
301 obtained for the cranberry pharmaceutical raw extract sample E3. Extracted ion
302 chromatogram and HRMS spectrum are also depicted in the figure.

303 Once all the fruit-based and pharmaceutical sample extracts were analyzed,
304 polyphenolic profiles were obtained by submitting the HRMS raw data to
305 ExactFinder^{TM} screening software and employing the user target accurate mass database
306 list of the 53 characterized polyphenols previously commented. To simplify the
307 obtained data, a threshold signal of 1.0E5 was set in the screening software to consider
308 that a compound could be present in the sample. Moreover, several confirmation
309 parameters such as accurate mass measurements (mass errors lower than 5 ppm),
310 isotopic pattern matches (higher than 85%), product ion scan spectra, and
311 chromatographic retention times were established. After raw data processing with
312 ExactFinder^{TM} software a report is provided for each sample depicting the peak areas of
313 all the targeted polyphenols found in agreement with the established confirmation
314 criteria (Table 2S in the supporting information shows the ExactFinder^{TM} report
315 obtained for the cranberry pharmaceutical raw extract sample E3).

316 UHPLC-HRMS polyphenolic profiles consisting of peak areas extracted by
317 ExactFinder^{TM} software in the fruit-based, pharmaceutical samples and QCs were then
318 obtained.

319

320 **Exploratory principal component analysis**

A data matrix containing the peak area information of the UHPLC-HRMS polyphenols of all analyzed samples was built to PCA exploration. The dimension of this polyphenolic matrix was 106 samples × 53 variables. Data was autoscaled with respect to the overall polyphenolic signal to provide similar weighs to all the samples. Figure 3 shows the score plot of PC1 *vs* PC2. It should be commented that QCs (not shown in the figure) appeared grouped showing a good repeatability and robustness of the proposed method. As can be seen, PC1 and PC2 roughly explained 65% of the data variance and a very acceptable discrimination among sample groups depending on the fruit of origin was achieved. For example, grape-based samples are grouped at the bottom of the score plot clearly separated from the other types of samples by PC2. Among the other samples, classification seem to be more related with PC1. In general, clear groups can be distinguished among them with the exception of some blueberry-based samples that are clustered together with some of the cranberry-based samples. Anyway, cranberry fruit samples are clearly discriminated from the raspberry ones. An interesting behavior was observed with the analyzed cranberry pharmaceutical samples. Those manufactured as sachets and syrups were grouped together with cranberry-fruit samples, while raw cranberry pharmaceutical extracts and capsules were completely discriminated and perfectly separated.

To better study this behavior and taking into consideration the raspberry, blueberry and grape extracts are expected to be used as adulterants of cranberry extracts, as previously commented in the introduction section, independent PCA models between cranberry-based samples and the other three fruit families studied were evaluated. Figure 4 shows the score and loading plots of (a) PC1 *vs* PC2 for cranberry- and raspberry-based samples, (b) PC2 *vs* PC3 for cranberry- and blueberry-based samples, and (c) PC1 *vs* PC2 for cranberry- and grape-based samples. As can be seen, cranberry-

346 based samples can be clearly differentiated, in general, from the other types of fruits,
347 showing that the UHPLC-HRMS profiling approach can be proposed as a useful
348 method to achieve the characterization and classification of the analyzed samples, as
349 well as for the authentication of fruit extracts regarding the type of fruit employed. By
350 analyzing the fruit extracts in pairs, the three PCA models showed that cranberry-based
351 pharmaceuticals can be clearly distinguished in three groups: capsules and extracts,
352 syrups and sachets, being the latest the ones that are in the three cases grouped close to
353 the cranberry-based fruit samples. It should be mentioned that when the study was
354 performed against blueberry-based samples (Figure 4b), capsules and extracts were
355 differentiated into three groups although none of them can be attributed only to either
356 capsule nor sachet presentation formats. The great differences between the cranberry-
357 based fruit samples with some of the cranberry-based pharmaceuticals (mainly syrups,
358 capsules and extracts) are clearly attributed to compositional differences associated to
359 the technological treatment to produce such products. It has been found that
360 concentration levels of the studied polyphenols are much higher in the pharmaceuticals
361 since raw materials are subjected to purification and preconcentration processes. Hence,
362 quantitative differences are partly compensated by data autoscaling although qualitative
363 differences due to the enrichment in active components occurring in the
364 pharmaceuticals are displayed in the PCA model. This finding was attributed to the fact
365 that the purification and preconcentration procedures followed by pharmaceutical
366 companies in the preparation of raw extracts from cranberry-fruits enhanced the
367 presence of some polyphenols in comparison to non-treated cranberry-fruit samples.
368 Loading plots revealed those polyphenols contributing more to the
369 discrimination of the samples. In general terms, it can be said that polyphenols such as
370 procyanidin A2, with A-type bonds, are clearly enhanced in some cranberry

pharmaceuticals such as capsules, extracts and syrups, fact which was reasonably expected as the extract purification and enrichment was focused on increasing the proportion of oligomeric PACs with respect to more simple compounds (for the same reason, procyanidin C1 and B2 were also in this part of the loading plot). Caffeic and coumaric acids were other components displaying higher proportions in the nutraceuticals. On the contrary, in the untreated cranberry-based samples comprising fresh fruits and raisins, homogentisic, sinapic and vanillic acids seemed to be abundant. Differences in the composition among raspberry and cranberry, and among blueberry and cranberry fruits were not so noticeable. More remarkable seemed to be the differences in the polyphenolics of cranberry with respect to grape, being the last class richer in gallic acid and quercetin.

Adulteration prediction by partial least square regression

The applicability of UHPLC-HRMS polyphenolic profiles for the authentication and quantitation of fraud levels of adulterant fruit extracts by PLS was also evaluated. For that purpose, cranberry-fruit extracts were adulterated with extracts of the other three fruits (blueberry, raspberry and grapes) at different concentration levels (2, 2.5, 5, 6, 7, 12, 20 and 50%). Triplicates of all the adulterations as well as of 100% pure fruit extracts were prepared. 50% adulteration was prepared in quintuplicate to evaluate data reproducibility. All sample extracts were then processed with the proposed sample treatment procedure and extract solutions analyzed with the UHPLC-HRMS method to obtain the polyphenolic profiles as previously explained. The calibration set (Table 1S in supporting information) was first employed to establish the PLS model as indicated in the experimental section. Venetian blinds cross validation method, considering 3 data splits, was used to estimate the number of latent variables (LV) used for the method

assessment. The performance of both calibration and prediction steps to predict adulterant percentages was studied under the selected model conditions. Figure 5 shows, as an example, the results obtained after applying the established PLS model for the prediction of grape adulterant levels in a cranberry fruit extract. Calibration and prediction errors obtained in all the adulteration cases studied are given in Table 3. As can be seen, very good quantitation of adulterant contents was obtained, with calibration errors in all cases below 0.01%, and prediction errors in the range of 2.71-5.96%. It should be considered that the proposed PLS models were evaluated for predicting values of low adulteration levels (2.5, 6 and 12%), demonstrating the appropriate performance of the developed method.

The results obtained in this work demonstrate that UHPLC-HRMS polyphenolic profiles by a simple screening of a home-made accurate mass database can be employed to achieve the characterization, classification and authentication of cranberry-based products and pharmaceuticals adulterated with more economic fruit-based extracts. HRMS provided, moreover, high selectivity and confirmation power to identify polyphenolic bioactive compounds that can be proposed as future biomarkers to address authentication issues of natural food-based products.

Conflict of Interest

There are no conflicts of interest to declare.

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Supporting Information description: Table S1: Samples Employed in the Training (Calibration) and Test (Prediction and Validation) Sets for Partial Least Squares Regression; Table S2: ExactFinderTM Report for the Cranberry Pharmaceutical Raw Extract Sample E3.

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588 **Figure captions**

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590 **Figure 1.** (a) HRMS spectrum, (b) MS/HRMS spectrum and (c) fragmentation pathway
591 of rutin.

592 **Figure 2.** UHPLC-HRMS total ion chromatogram (TIC) for cranberry pharmaceutical
593 raw extract sample E3, and extracted ion chromatogram and HRMS spectrum of
594 procyanidin A2 in the same sample.

595 **Figure 3.** PCA score plot of PC1 *vs* PC2 obtained using UHPLC-HRMS polyphenolic
596 profiles of all the analyzed samples.

597 **Figure 4.** PCA score and loading plots of (a) PC1 *vs* PC2 for cranberry- and raspberry-
598 based samples, (b) PC2 *vs* PC3 for cranberry- and blueberry-based samples, and (c) PC1
599 *vs* PC2 for cranberry- and grape-based samples.

600 **Figure 5.** PLS model applied to the quantitation of the grape percentage on cranberry-
601 fruit extracts adulterated when using UHPLC-HRMS polyphenolic profiles.

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Table 1. Chemical Structures and Classification of the Studied Polyphenols.

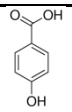
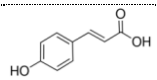
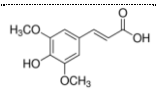
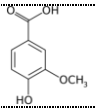
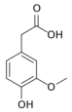
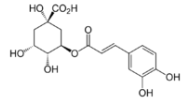
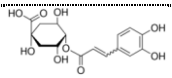
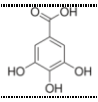
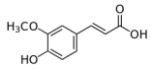
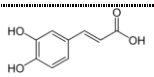
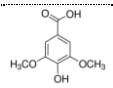
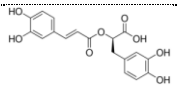
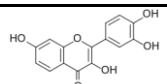
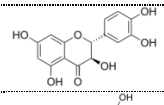
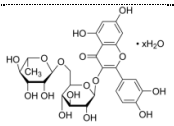
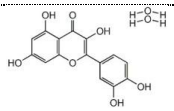
Compounds	Formula	CAS number	Structure
Phenolic acids			
4-Hydroxybenzoic acid	$C_7H_6O_3$	99-96-7	
p-Coumaric acid	$C_9H_8O_3$	501-98-4	
Sinapic acid	$C_{11}H_{12}O_5$	530-59-6	
Vanillic acid	$C_8H_8O_4$	121-34-6	
Homovanillic acid	$C_9H_{10}O_4$	306-08-1	
Homogentisic acid	$C_8H_8O_4$	451-13-8	
Chlorogenic acid	$C_{16}H_{18}O_9$	327-97-9	
Cryptochlorogenic acid	$C_{16}H_{18}O_9$	905-99-7	
Gallic acid	$C_7H_6O_5$	149-91-7	
Ferulic acid	$C_{10}H_{10}O_4$	537-98-4	
Gentisic acid	$C_7H_6O_4$	490-79-9	
Caffeic acid	$C_9H_8O_4$	331-39-5	
Syringic acid	$C_9H_{10}O_5$	530-57-4	
Rosmarinic acid	$C_{18}H_{16}O_8$	20283-92-5	
Flavones			
Fisetin	$C_{15}H_{10}O_6$	528-48-3	
Taxifolin	$C_{15}H_{12}O_7$	480-18-2	
Rutin	$C_{27}H_{30}O_{16}$	207671-50-9	
Quercetin	$C_{15}H_{14}O_9$	6151-25-3	

Table 1. Chemical Structures and Classification of the Studied Polyphenols (continuation).

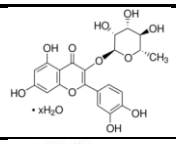
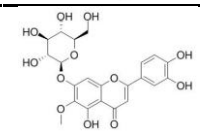
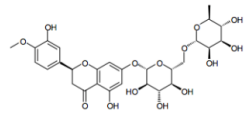
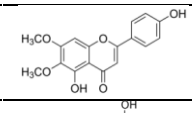
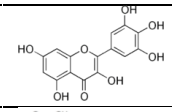
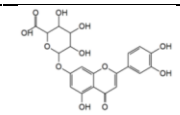
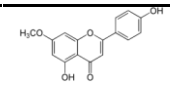
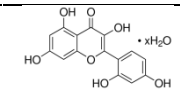
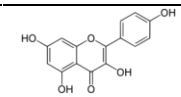
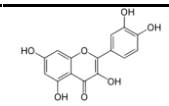
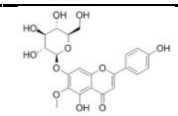
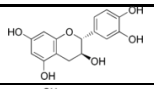
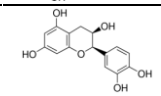
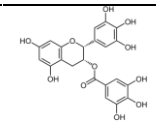
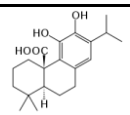
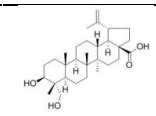
Compounds	Formula	CAS number	Structure
Quercitrin	$C_{21}H_{20}O_{11}$	522-12-3	
Nepetin-7-glucoside	$C_{22}H_{22}O_{12}$	569-90-4	
Hesperidin	$C_{28}H_{34}O_{15}$	520-26-3	
Cirsimaritin	$C_{17}H_{14}O_6$	6601-62-3	
Myricetin	$C_{15}H_{10}O_8$	529-44-2	
Luteolin-7-O-β-d-glucuronide	$C_{21}H_{18}O_{12}$	38934-20-2	
Genkwanin	$C_{16}H_{12}O_5$	437-64-9	
Morin	$C_{15}H_{10}O_7$	654055-01-3	
Kaempferol	$C_{15}H_{10}O_6$	520-18-3	
Quercetin	$C_{15}H_{10}O_7$	117-39-5	
Homoplantaginin	$C_{22}H_{22}O_{11}$	17680-84-1	
Flavanols			
(+)-Catechin	$C_{15}H_{14}O_6$	7295-85-4	
(-)-Epicatechin	$C_{15}H_{14}O_6$	490-46-0	
(-)-Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	989-51-5	
Phenolic terpenes			
Carnosic acid	$C_{20}H_{28}O_4$	3650-09-07	
Anemosapogenin	$C_{30}H_{48}O_4$	85999-40-2	

Table 1. Chemical Structures and Classification of the Studied Polyphenols (continuation).

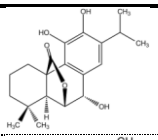
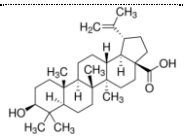
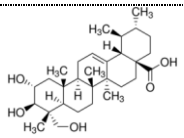
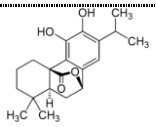
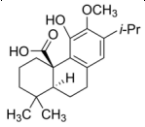
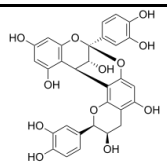
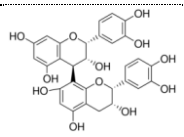
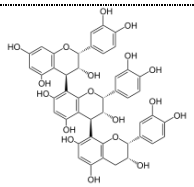
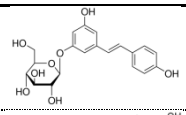
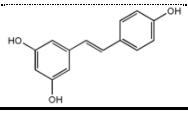
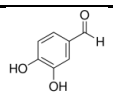
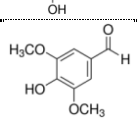
Compounds	Formula	CAS number	Structure
Rosmanol	$C_{20}H_{26}O_5$	80225-53-2	
Betulinic acid	$C_{30}H_{46}O_3$	472-15-1	
Asiatic acid	$C_{30}H_{48}O_5$	464-92-6	
Carnosol	$C_{20}H_{26}O_4$	5957-80-2	
12-methoxycarnosic acid	$C_{21}H_{30}O_4$	3650-09-07	
Proanthocyanidins			
Procyanidin A2	$C_{30}H_{24}O_{12}$	41743-41-3	
Procyanidin B2	$C_{30}H_{26}O_{12}$	29106-49-8	
Procyanidin C1	$C_{45}H_{38}O_{18}$	37064-30-5	
Stilbenes			
Polydatin	$C_{20}H_{22}O_8$	65914-17-2	
Resveratrol	$C_{14}H_{12}O_3$	501-36-0	
Phenolic aldehydes			
3,4-dihydroxybensaldehyde	$C_7H_6O_3$	139-85-5	
Syringaldehyde	$C_9H_{10}O_4$	134-96-3	

Table 1. Chemical Structures and Classification of the Studied Polyphenols (continuation).

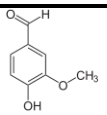
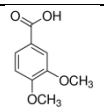
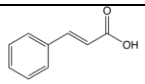
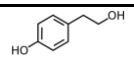
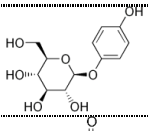
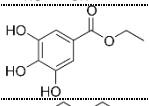
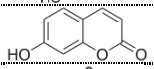
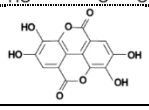
Compounds	Formula	CAS number	Structure
Vanillin	$C_8H_8O_3$	121-33-5	
Benzoic acids			
Veratric acid	$C_9H_{10}O_4$	93-07-2	
Cinnamic acids			
trans-Cinnamic acid	$C_9H_8O_2$	140-10-3	
Other Phenolics			
Tyrosol	$C_8H_{10}O_2$	501-94-0	
Arbutin	$C_{12}H_{16}O_7$	497-76-7	
Ethyl gallate	$C_9H_{10}O_5$	831-61-8	
Umbelliferon	$C_9H_6O_3$	93-35-6	
Ellagic acid	$C_{14}H_6O_8$	746-66-4	

Table 2. HRMS and MS/HRMS (Product Ion Spectra) of the Studied Polyphenolic Compounds.

Compounds	RT (min)	Chemical formula	HRMS spectrum			MS/HRMS spectrum		
			[M-H]$^-$ *m/z* calculated value	[M-H]$^-$ *m/z* experimental value	Accurate mass error (ppm)	Fragment ions (*m/z*)	Assignment	Accurate mass error (ppm)
Phenolic acids								
4-Hydroxybenzoic acid	4.1	$C_7H_6O_3$	137.02442	137.02428	-1.022	93.03453	[M-H-COO]$^-$	-0.626
p-Coumaric acid	5.8	$C_9H_8O_3$	163.04007	163.04005	-0.123	119.05017	[M-H-COO]$^-$	-0.573
Sinapic acid	6.2	$C_{11}H_{12}O_5$	223.0612	223.06089	-1.390	208.03748	[M-H-CH_3]$^{-\cdot}$	-1.161
						193.01442	[M-H-C_2H_6]$^-$	0.898
Vanillic acid	4.6	$C_8H_8O_4$	167.03498	167.03487	-0.659	152.01118	[M-H-CH_3]$^-$	-2.150
						123.04528	[M-H-COO]$^-$	1.034
						108.02161	[M-H-$C_2H_3O_2$]$^{-\cdot}$	-0.627
Homovanillic acid	4.9	$C_9H_{10}O_4$	181.05063	181.05076	0.718	--	--	--
Homogentisic acid	2.1	$C_8H_8O_4$	167.03498	167.03485	-0.778	123.04506	[M-H-COO]$^-$	-0.754
Chlorogenic acid	3.8	$C_{16}H_{18}O_9$	353.08781	353.08782	0.028			
Cryptochlorogenic acid	4.2	$C_{16}H_{18}O_9$	353.08781	353.08795	0.397	191.05617	[M-H-$C_9H_6O_3$]$^-$	0.307
						179.03503	[M-H-$C_7H_{10}O_5$]$^-$	0.268
						173.04546	[M-H-$C_9H_8O_4$]$^-$	-0.501
						135.04512	[M-H-$C_8H_{10}O_7$]$^-$	-0.243
Gallic acid	1.5	$C_7H_6O_5$	169.01425	169.01428	0.177	125.02431	[M-H-COO]$^-$	-0.858
Ferulic acid	6.3	$C_{10}H_{10}O_4$	193.05063	193.05073	0.518	134.03723	[M-H-$C_2H_3O_2$]$^{-\cdot}$	-0.730
Gentisic acid	4.3	$C_7H_6O_4$	153.01933	153.01919	-0.915	109.02942	[M-H-COO]$^-$	-0.759
Caffeic acid	4.7	$C_9H_8O_4$	179.03498	179.03474	-1.341			
Syringic acid	4.8	$C_9H_{10}O_5$	197.04555	197.04568	0.660			
Rosmarinic acid	7.2	$C_{18}H_{16}O_8$	359.07724	359.07722	-0.058	197.04576	[M-H-$C_9H_6O_3$]$^-$	1.082
						179.03477	[M-H-$C_9H_8O_4$]$^-$	-1.184
						161.02420	[M-H-$C_9H_{10}O_5$]$^-$	-1.350
						135.04501	[M-H-$C_{10}H_8O_6$]$^-$	-1.057
						123.04506	[M-H-$C_{11}H_8O_6$]$^-$	-0.133
						72.99301	[M-H-$C_{16}H_{14}O_5$]$^{-\cdot}$	-1.469
Flavones								
Fisetin	7.4	$C_{15}H_{10}O_6$	285.04046	285.0463	0.596	229.05011	[M-H-C_2O_2]$^-$	-2.279
						163.00324	[M-H-$C_7H_6O_2$]$^-$	-2.711
Taxifolin	6.5	$C_{15}H_{12}O_7$	303.05103	303.05120	0.561			
Rutin	5.8	$C_{27}H_{30}O_{16}$	609.14611	609.14665	0.886	301.03543	[M-H-$C_{12}H_{20}O_9$]$^-$	0.180
						300.02759	[M-H-$C_{12}H_{21}O_9$]$^{-\cdot}$	0.131
						271.02469	[M-H-$C_{13}H_{22}O_{10}$]$^-$	-0.447
						255.02975	[M-H-$C_{13}H_{22}O_{11}$]$^-$	-0.575
						243.02921	[M-H-$C_{14}H_{22}O_{11}$]$^-$	-2.825
Quercitrin	6.7	$C_{21}H_{20}O_{11}$	447.09328	447.09338	0.224	301.03551	[M-H-$C_6H_{10}O_4$]$^-$	0.445
						300.02746	[M-H-$C_6H_{11}O_4$]$^{-\cdot}$	-0.303
						271.02432	[M-H-$C_7H_{12}O_5$]$^-$	-1.812
						255.02910	[M-H-$C_7H_{12}O_{10}$]$^-$	-3.124
						151.00333	[M-H-$C_{14}H_{16}O_7$]$^-$	-2.330
Nepetin-7-glucoside	6.3	$C_{22}H_{22}O_{12}$	477.10385	477.10381	-0.084	327.05170	[M-H-$C_5H_{10}O_5$]$^-$	2.061
						299.01971	[M-H-$C_7H_{14}O_5$]$^-$	-0.053
Hesperidin	6.8	$C_{28}H_{34}O_{15}$	609.18249	609.18272	0.378	301.07175	[M-H-$C_{12}H_{20}O_9$]$^-$	3.605
Cirsimaritin	11.2	$C_{17}H_{14}O_6$	313.07176	313.07191	0.479	283.02478	[M-H-C_2H_6]$^-$	-0.110
Myricetin	4.7	$C_{15}H_{10}O_8$	317.03029	317.03037	0.252			
Luteolin-7-O-β-d-glucuronide	6.2	$C_{21}H_{18}O_{12}$	461.07255	461.07282	0.586	285.04047	[M-H-$C_6H_8O_6$]$^-$	0.031
Genkwanin	12.5	$C_{16}H_{12}O_5$	283.06120	283.06119	-0.035	268.03782	[M-H-CH_3]$^-$	0.367
Morin	8.0	$C_{15}H_{10}O_7$	301.03528	301.03530	-0.266	151.00340	[M-H-$C_8H_6O_3$]$^-$	-1.867
						148.01627	[M-H-$C_7H_5O_4$]$^{-\cdot}$	-2.177
						125.02435	[M-H-$C_9H_4O_4$]$^-$	-0.538
						107.01381	[M-H-$C_9H_6O_5$]$^-$	-0.399
						83.01386	[M-H-$C_{11}H_6O_5$]$^-$	0.088
Kaempferol	9.9	$C_{15}H_{10}O_6$	285.04046	285.04047	0.035			
Quercetin	6.5	$C_{15}H_{10}O_7$	301.03538	301.03534	-0.133			

Homoplantaginin	6.9	$C_{22}H_{22}O_{11}$	461.10893	461.10912	0.402	283.02475	$[M-H-C_7H_{14}O_5]^-$	-0.216
Flavones								
(+)-Catechin	4.0	$C_{15}H_{14}O_6$	289.07176	289.07181	0.173	245.08175	$[M-H-COO]^-$	3.732
						203.07145	$[M-H-C_3H_2O_3]^-$	0.406
						123.04513	$[M-H-C_8H_6O_4]^-$	-0.185
						109.02938	$[M-H-C_9H_8O_4]^-$	-1.125
(-)-Epicatechin	4.9	$C_{15}H_{14}O_6$	289.07176	289.07181	0.173	245.08160	$[M-H-COO]^-$	-1.355
						203.07156	$[M-H-C_3H_2O_3]^-$	0.948
						123.04504	$[M-H-C_8H_6O_4]^-$	-0.917
						109.02942	$[M-H-C_9H_8O_4]^-$	-0.759
(-)-Epigallocatechin gallate	5.5	$C_{22}H_{18}O_{11}$	457.07763	457.07729	-0.744			
Phenolic terpenes								
Carnosic acid	17.1	$C_{20}H_{28}O_4$	331.19148	331.19145	-0.091	287.20172	$[M-H-COO]^-$	0.232
Anemosapogenin	15.5	$C_{30}H_{48}O_4$	471.34798	471.34788	-0.212			
Rosmanol	11.8	$C_{20}H_{26}O_5$	345.17075	345.17062	-0.377			
Betulinic acid	20.0	$C_{30}H_{46}O_3$	455.35307	455.35318	0.245			
Asiatic acid	12.5	$C_{30}H_{48}O_5$	487.34290	487.34293	0.062			
Carnosol	15.2	$C_{20}H_{26}O_4$	329.17583	329.17599	0.486	285.18607	$[M-H-COO]^-$	0.234
12-methoxycarnosic acid	18.2	$C_{21}H_{30}O_4$	345.20713	345.20695	-0.521	301.21722	$[M-H-COO]^-$	-0.277
						286.19376	$[M-H-C_2H_3O_2]^{-\cdot}$	-0.239
Proanthocyanidins								
Procyanidin A2	6.5	$C_{30}H_{24}O_{12}$	575.11950	575.11996	0.800	285.04068	$[M-H-C_{15}H_{14}O_6]^-$	0.767
Procyanidin B2	2.7	$C_{30}H_{26}O_{12}$	577.13515	577.13525	0.173			
Procyanidin C1	5.1	$C_{45}H_{38}O_{18}$	865.19854	865.19998	1.664			
Stilbenes								
Polydatin	7.0	$C_{20}H_{22}O_8$	389.12419	389.12450	0.797	227.07114	$[M-H-C_6H_{10}O_5]^-$	-1.002
Resveratrol	8.9	$C_{14}H_{12}O_3$	227.07137	227.07140	0.132			
Phenolic aldehydes								
3,4-dihydroxybensaldehyde	3.9	$C_7H_6O_3$	137.02442	137.02413	-2.116			
Syringaldehyde	6.0	$C_9H_{10}O_4$	181.05063	181.05073	0.552			
Vanillin	5.8	$C_8H_8O_3$	151.04007	151.03984	-1.506			
Benzoic acids								
Veratric acid	7.6	$C_9H_{10}O_4$	181.05063	181.05065	0.110			
Cinnamic acids								
Trans-Cinnamic acid	9.2	$C_9H_8O_2$	147.04515	147.04525	0.680			
Other Phenolics								
Tyrosol	4.9	$C_8H_{10}O_2$	137.06080	137.06071	-0.657			
Arbutin	1.3	$C_{12}H_{16}O_7$	271.08233	271.08229	-0.148	108.02164	$[M-H-C_6H_{11}O_5]^{-\cdot}$	-0.349
Ethyl gallate	5.9	$C_9H_{10}O_5$	197.04555	197.04542	-0.660	169.01402	$[M-H-C_2H_4]^-$	-1.341
						124.01646	$[M-H-C_3H_5O_2]^-$	-1.067
Umbelliferon	6.3	$C_9H_6O_3$	161.02442	161.02438	-0.248			
Ellagic acid	6.0	$C_{14}H_6O_8$	300.99899	300.99901	0.066			

Table 3. Prediction Errors by PLS Regression in the Quantification of Cranberry-fruit Extracts Adulterated with Raspberry-, Blueberry-, and Grape-fruit Extracts.

Adulterant	Number of latent variables	Calibration error	Prediction error
Grape	3	<0.01%	2.86%
Blueberry	3	<0.01%	2.71%
Raspberry	3	<0.01%	5.96%

Figure 1

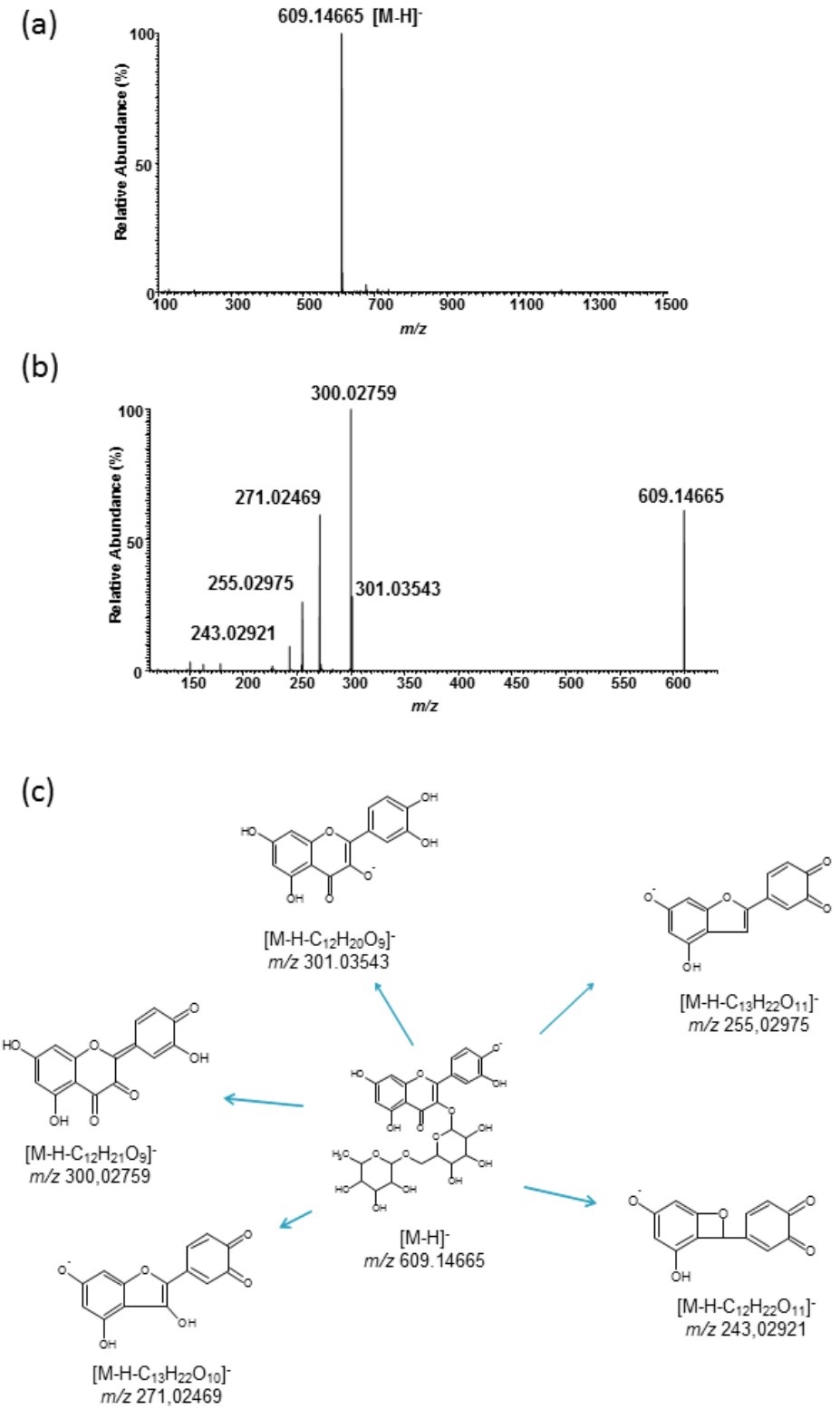

(a)
609.14665 [M-H]-
Relative Abundance (%)
m/z
(b)
300.02759
271.02469
609.14665
255.02975
301.03543
243.02921
Relative Abundance (%)
m/z
(c)
[M-H-C12H20O9]-
m/z 301.03543
[M-H-C13H22O11]-
m/z 255,02975
[M-H-C12H21O9]-
m/z 300,02759
[M-H]-
m/z 609.14665
[M-H-C12H22O11]-
m/z 243,02921
[M-H-C13H22O10]-
m/z 271,02469

Figure 2

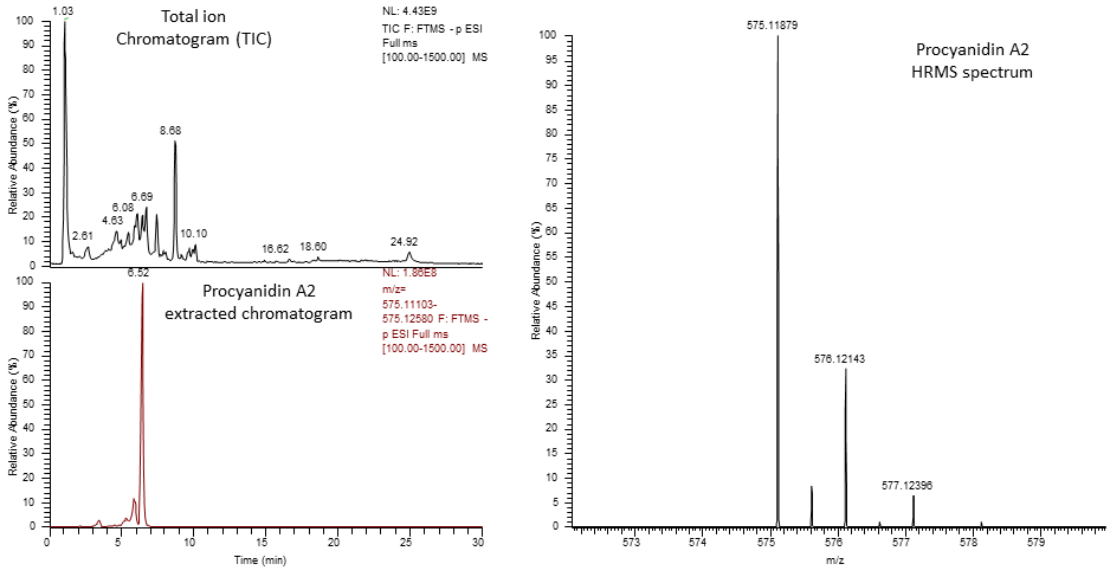

Total ion
Chromatogram (TIC)
NL: 4.43E9
TIC F: FTMS - p ESI
Full ms
[100.00-1500.00] MS
1.03
2.61
4.63
6.08
6.69
8.68
10.10
16.62
18.60
24.92
Procyanidin A2
extracted chromatogram
NL: 1.88E8
m/z=
575.11103-
575.12580 F: FTMS -
p ESI Full ms
[100.00-1500.00] MS
6.52
Relative Abundance (%)
Time (min)
Procyanidin A2
HRMS spectrum
575.11879
576.12143
577.12396
m/z

Figure 3

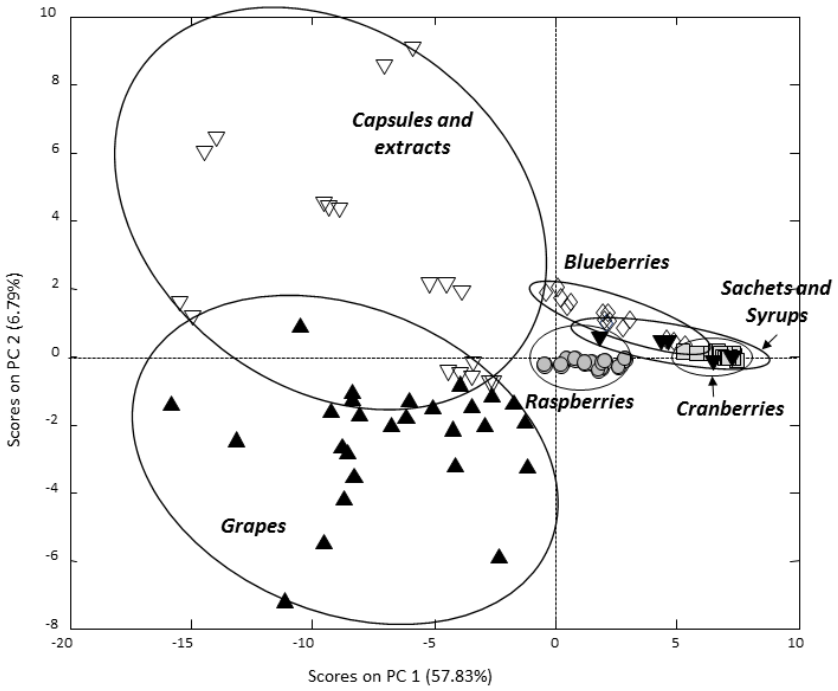

Capsules and extracts
Blueberries
Sachets and Syrups
Raspberries
Cranberries
Grapes
Scores on PC 2 (6.79%)
Scores on PC 1 (57.83%)
10
8
6
4
2
0
-2
-4
-6
-8
-20
-15
-10
-5
0
5
10

Figure 4

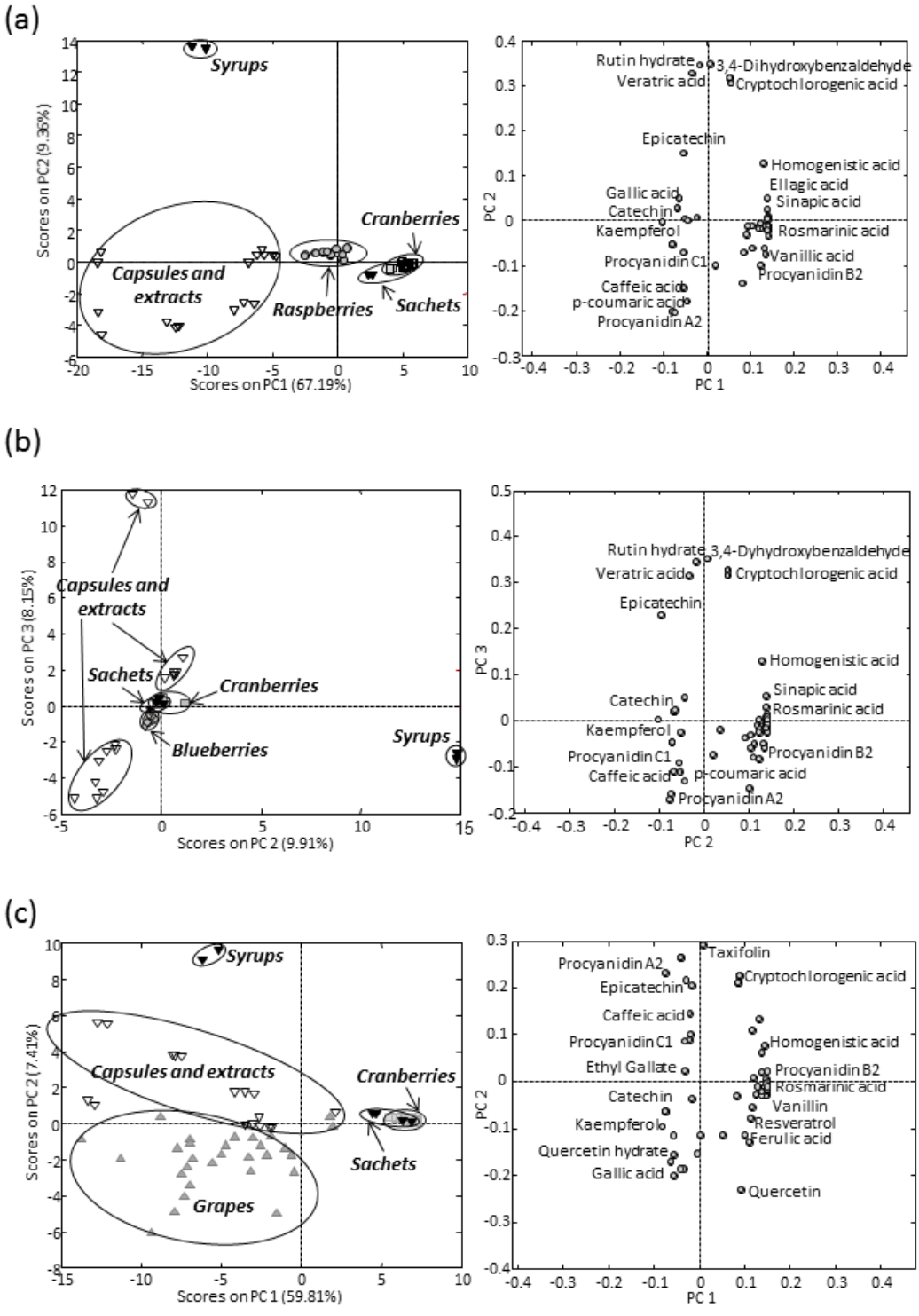
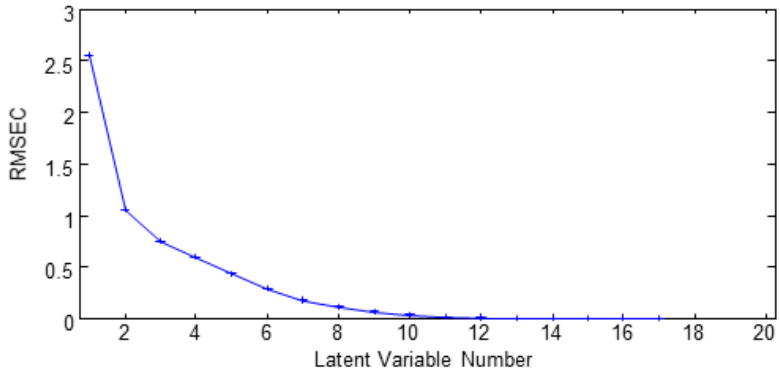
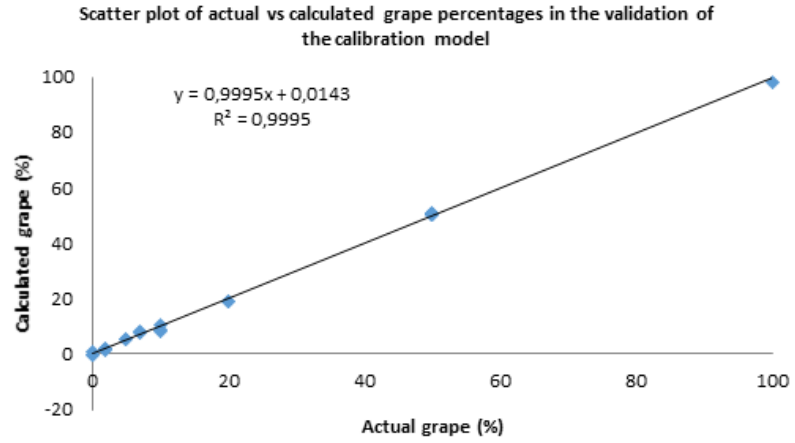
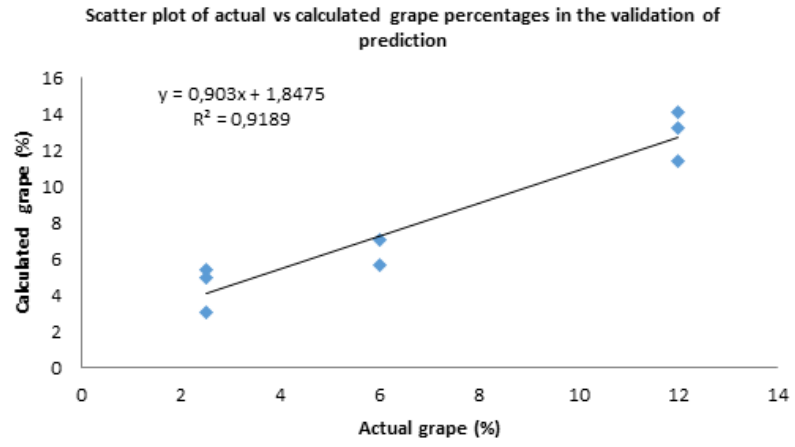

(a)
Syrups
Cranberries
Capsules and extracts
Raspberries
Sachets
Scores on PC2 (9.36%)
Scores on PC1 (67.19%)
Rutin hydrate
3,4-Dihydroxybenzaldehyde
Veratric acid
Cryptochlorogenic acid
Epicatechin
Homogenistic acid
Ellagic acid
Gallic acid
Sinapic acid
Catechin
Kaempferol
Rosmarinic acid
Procyanidin C1
Vanillic acid
Procyanidin B2
Caffeic acid
p-coumaric acid
Procyanidin A2
PC 2
PC 1
(b)
Capsules and extracts
Sachets
Cranberries
Blueberries
Syrups
Scores on PC 3 (8.15%)
Scores on PC 2 (9.91%)
Rutin hydrate
3,4-Dyhydroxybenzaldehyde
Veratric acid
Cryptochlorogenic acid
Epicatechin
Homogenistic acid
Sinapic acid
Catechin
Rosmarinic acid
Kaempferol
Procyanidin C1
Procyanidin B2
Caffeic acid
p-coumaric acid
Procyanidin A2
PC 3
PC 2
(c)
Syrups
Capsules and extracts
Cranberries
Sachets
Grapes
Scores on PC 2 (7.41%)
Scores on PC 1 (59.81%)
Taxifolin
Procyanidin A2
Cryptochlorogenic acid
Epicatechin
Caffeic acid
Homogenistic acid
Procyanidin C1
Ethyl Gallate
Procyanidin B2
Rosmarinic acid
Catechin
Vanillin
Kaempferol
Resveratrol
Ferulic acid
Quercetin hydrate
Gallic acid
Quercetin
PC 2
PC 1

Figure 5


RMSEC
Latent Variable Number


Scatter plot of actual vs calculated grape percentages in the validation of the calibration model
y = 0,9995x + 0,0143
R² = 0,9995
Calculated grape (%)
Actual grape (%)


Scatter plot of actual vs calculated grape percentages in the validation of prediction
y = 0,903x + 1,8475
R² = 0,9189
Calculated grape (%)
Actual grape (%)

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