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Liquid Chromatographic Fingerprints for the Characterization of Flavanol-Rich Nutraceuticals Based on 4-Dimethylaminocinnamaldehyde Precolumn Derivatization

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Abstract: Flavanols consist of a great family of bioactive molecules displaying a wide range of health-promoting attributes for humans, including antioxidant, antimicrobial or anti-inflammatory effects. As a result, botanical species rich in this type of compound are often used to develop nutraceutical products or dietary supplements with recognized healthy attributes. This paper aims at characterizing nutraceutical products using liquid chromatographic fingerprints related to flavanol composition. Catechins and their oligomers were exploited to characterize and authenticate various commercial products prepared with extracts of red berries and medicinal plants. These compounds resulted in interesting descriptors of some fruits and vegetables, thus providing an additional perspective for the study of nutraceuticals. For such a purpose, a new method based on liquid chromatography with UV/Vis detection (HPLC–UV/Vis) with precolumn derivatization with 4-dimethylaminocinnamaldehyde was developed. Results indicated that the separation of flavanols was very complex due to the degradation of procyanidin derivatives. The resulting data sets were analyzed using chemometric methods such as principal component analysis and partial least square–discriminant analysis. Despite the complexity of chromatographic fingerprints, nutraceutical samples could be discriminated according to their main ingredients. In general, catechin and epicatechin were the most abundant compounds in the different samples, and procyanidin A_2 was highly specific to cranberry.

Keywords: red berries; medicinal plants; flavonoids; nutraceuticals; 4-dimethylaminocinnamaldehyde; HPLC–UV; principal component analysis; partial least square–discriminant analysis

1. Introduction

The consumption of nutraceutical products and functional foods has experienced enormous growth in recent years due to its crucial role in the prevention of some diseases and the positive effects for human health associated with the bioactive compounds that they contain. Nowadays, the pharmaceutical industry produces nutraceuticals made from plants, and some remarkable examples are given as follows. For instance, American cranberry (*Vaccinium macrocarpon*) has a wide range of beneficial components, such as vitamins, β-carotenes, organic acids, phenolic acids, and flavonoids [1–5]. In particular, the latter display well-known antioxidant, antimicrobial, anti-inflammatory, antineoplastic and cardioprotective attributes [6–8]. Other commercial products are prepared with raspberry (*Rubus idaeus*) and green tea (*Camellia sinensis*) extracts, rich in phenolic ketones and flavonoids, such as epigallocatechin gallate, that may contribute to reducing weight or control obesity [9]. Grape-based supplements also contain remarkable amounts of polyphenolic antioxidants to neutralize the excess of free radicals [10]. Among them, stilbenes

(resveratrol and its derivatives) stand out because of their high antioxidant power and their controversial antiaging activity. Artichoke is another recognized product with a great range of bioactive compounds (phenolic acids, sesquiterpenes, and flavonoids) with antispasmodic, antiemetic, and anticholesterolemic properties [11]. Currently, some trendy dietary supplements consist of varied mixtures of fruit and medicinal plant extracts with exceptional antioxidant features.

As can be inferred, the aforementioned nutraceutical products are used as a remarkable source of flavonoids, highlighting the procyanidins (PACs, also known as nonhydrolyzable tannins) that are polymers of flavanols. Monomers can be linked via B-type bonds connecting the C4 of the upper unit with the C6 or C8 of the lower monomer, or A-type bonds with oxygen linking the C2 of the upper monomer and the hydroxyl group of C5 o C7 of the lower monomer (i.e., C2–O–C5 or C2–O–C7). The structure of some important flavanols is presented in Figure 1. In nature, B-type compounds are more abundant than A-type ones. Regardless, A-type compounds possess great antimicrobial activity so that they are very useful for the prevention and treatment of urinary tract infections (UTIs) such as cystitis [12–15].
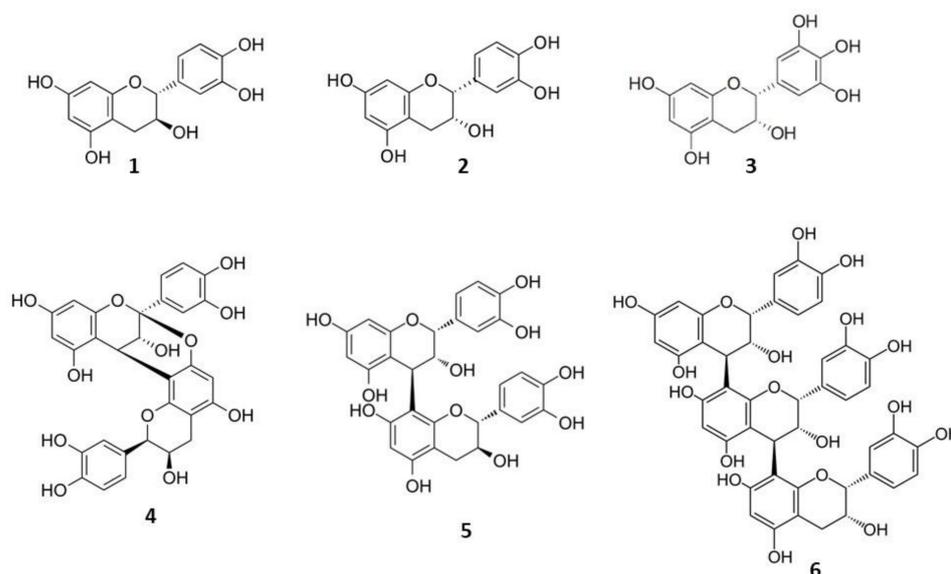

Figure 1. Scheme of some relevant flavanols. 1 = catechin; 2 = epicatechin; 3 = epigallocatechin; 4 = procyanidin A2; 5 = procyanidin B1; 6 = procyanidin C1.

Currently, the 4-dimethylaminocinnamaldehyde (DMAC) spectrophotometric assay is the most popular method used for the quantification of overall PAC contents in nutraceuticals and functional foods. Alternative methods such as the vanillin–acid assay and HCl–butanol seem to be more limited [16,17]. DMAC is a highly selective reagent towards flavanols, giving strongly colored derivatives that absorb at 640 nm (thus reducing the possible anthocyanin interference). The molar absorption coefficients of flavanols with DMAC tend to decrease as the polymerization degree increases, and therefore, monomers as catechin and epicatechin display higher sensitivity [18–21].

Apart from spectrophotometric indexes, a typical approach for the quantification and characterization of PACs relies on reversed phase high-performance liquid chromatography (RP–HPLC) with UV/Vis and fluorescence detection (FLD) [22–24]. For instance, Bakhytkyzy et al. compared both detection modes, concluding that FLD (excitation at 280 nm and emission at 320 nm) was more selective than UV/Vis at 280 nm [25]. Regardless, the determination of PACs is complex, especially when dealing with polymeric molecules. The RP mode does not allow the separation of PACs with more than three units [26]. In contrast, normal phase chromatography is more recommendable to deal with the separation of oligomers up to 10 units [27]. Alternatively, Zou et al. successfully used size

exclusion chromatography, providing similar results to the normal phase [28]. Hyphenated techniques with mass spectrometry (MS), such as HPLC–MS and HPLC–MS/MS, were applied to identify and quantify PACs and other related phenolic compounds [22,23,29–32].

Although natural extracts from medicinal plants have been extensively used for the preparation of pharmaceutical and nutraceutical products, it should be highlighted that their composition and richness of bioactive compounds strongly depend on agricultural practices and environmental conditions [33]. This issue has been evidenced in various remarkable reviews such as those from Yang et al., Heimler et al., and Lanza et al. [33–36]. Secondary plant metabolites such as flavonoids and phenolic acids play significant roles in plant defense mechanisms against plagues of insects and microorganisms, as well as in front of climatic or farming factors; hence, it is not surprising that their levels depend on the stress suffered by the plants. More recent specific examples to illustrate this dependence deal with the study of the antioxidant features and phenolic contents of blackberry extracts cultivated in different climates [37], anthocyanin levels of blueberry species as a function of ripening or altitude [38], essential oil, carotenoid, polyphenol composition of *Thymus* sp. under controlled growing conditions (e.g., temperature and humidity) [39], the compositional profiles of essential oils and polyphenols of various medicinal extracts of plants grown in different altitudes [40], as well as seasonal variations on bioactive compounds [41]. The natural variability in the composition of extracts is an important issue to be solved in order to correctly standardize the bioactivity of pharmaceutical or nutraceutical products elaborated from plant sources.

Some chemometric characterizations of nutraceuticals and fruit extracts based on the composition of phenolic acids and flavonoids showed that catechin, epicatechin, and quercitrin were markers of cranberry [22,23], meaning that their levels were up expressed in this type of samples with respect to other matrices. The study by Gardana et al. showed several effective markers of cranberry-based products, among them anthocyanins such as delphinidin glucoside, cyanidin rutinoside, delphinidin glucose-xylose, and cyanidin-glucose-xylose [42]. Moreover, the authors concluded that epicatechin was the main monomer in cranberry fruits and procyanidin A_2 the main dimer. Hurkova et al. studied the markers that differentiated two *Vaccinium* species (cranberries and lingonberries) from LC–HRMS/MS fingerprinting [43]. Discriminating features deduced from PLS–DA were tentatively elucidated from MS isotopic and MS/MS fragmentation patterns. The results showed that peonidin 3-*O*-arabinoside and myricetin 3-glucoside were absent in lingonberries. The compounds that most influenced the discrimination of lingonberry samples were catechin and ferulic acid.

The main aim of this research is the characterization of nutraceutical products made with cranberry, raspberry, grapevine, red grape, artichoke, and mixtures of berries with other plant ingredients, using the composition of flavanols as the source of analytical information. The extraction of flavanols in the nutraceutical fruit samples was carried out by ultrasound-assisted extraction (UAE) using an acidified hydro-organic solvent according to a previously established procedure [25,44]. The chromatographic method used was HPLC–UV/Vis with precolumn flavanol derivatization with DMAC. The optimal separation conditions were chosen to provide the richest compositional profiles, i.e., looking for the resolution of the maximum number of compounds of the samples. Subsequently, samples were characterized according to their flavanol content using chemometric methods.

2. Materials and Methods

2.1. Reagents and Solutions

Procyanidin A_2 (>99%) and procyanidin C_1 (>99%) were purchased from Phytolab (Vestenbergsgreuth, Germany), procyanidin B_2 (>98%) from Chengdu Biopurify Phytochemicals (Chengdu, China), catechin (>98%), and epicatechin (>98%) from Sigma-Aldrich (St Louis, MO, USA), and epigallocatechin gallate (>98%) from Carbosynth (Berkshire, UK), and 4-dimethylaminocinnamaldehyde (>98%, DMAC) was from Tokyo Chemical Industry (Tokyo, Japan).

Other chemicals for the extraction, derivatization, and preparation of the mobile phases were hydrochloric acid (37%, Panreac, Barcelona, Spain), formic acid (≥95%, Sigma-Aldrich), methanol and ethanol (99.9%, UHPLC Supergradient, Panreac), and acetone (>99%, for analysis, Merck, Darmstadt, Germany). Purified water was generated with an Elix 3 coupled with a Mili-Q system (Bedford, MA, USA).

Individual standard solutions at 1000 mg L^{-1} each compound were prepared using methanol as the solvent. These stock standards were further diluted with H_2O/MeOH (75:25, *v/v*) to prepare the working solutions. The reagent solution for flavanol derivatization consisted of 0.16% (*w/v*) DMAC with 0.2 M HCl; this reagent was prepared daily and was kept in an amber vial at 4 °C.

2.2. Instruments and Apparatus

An Agilent Series 1100 HPLC chromatograph (Agilent, Technologies, Palo Alto, CA, USA) was used, equipped with a binary pump (G1312A), an autosampler (G1379A), a degasser system (G1379A), and a diode array (DAD, G1315B) and fluorescence (FLD, G1321A) detectors. The separation was carried out at 20 °C with a Kinetex C18 (100 mm length × 4.6 mm I.D, 2.6 μm particle size) with a C18 precolumn (4.00 mm length × 3.00 mm I.D) from Phenomenex (Torrance, CA, USA).

A Lambda19 double beam UV–Vis–NIR spectrophotometer (PerkinElmer, Waltham, MA, USA) was used for the DMAC spectrophotometric method. Measurements were performed with 10 mm path length cells (QS quartz glass, Hellma, Müllheim, Germany). The absorbance was recorded at 640 nm.

An IKA RCT basic heater (IKA-Werke, Staufen, Germany), a Genius 3 Vortex mixer (IKA, Staufen, Germany), a PB1502-L analytical balance (Mettler-Toledo, Columbus, OH, USA), a Branson 5510 ultrasonic bath (Branson Ultrasonic Corporation, Danbury, CT, USA) and a Rotanta 460 RS centrifuge (Hettich, Tuttlingen, Germany) were used for sample processing.

2.3. Samples

There is a wide variety of nutraceutical products in the market whose activities are mainly based on the composition of flavanols. A comprehensive study of this wide range of products is out of the scope and the possibilities of this paper; therefore, several representative examples were here selected as a proof of concept to explore the possibilities of the DMAC precolumn derivatization method. In particular, nutraceuticals under study were prepared with American cranberry, raspberry and green tea, grape products, artichoke, and pomegranate as the principal ingredients. These extracts were widely used because of their antimicrobial, sliming, cardioprotective, hypolipidemic, and antioxidant attributes. Overall, 21 commercial nutraceutical samples were purchased from herbalist shops in Barcelona (Spain) and Gdańsk (Poland). Table 1 summarizes the principal characteristic of these samples regarding manufacturers, composition, and attributed activities.

Table 1. List of samples under study with some relevant features.

Sample Type	Product Name	Manufacturing co.	Pharmaceutical Form	Composition	Indications
Cranberry (1)	Aquilea Cistitus	Uriach (Spain)	Tablet	*Vaccinium macrocarpon*	Urinary tract health, cystitis,
Cranberry (2)	Żurawina	Colfarm (Poland)	Tablet	*Vaccinium macrocarpon, Urtica urens*	Urinary tract health, cystitis, diuretic
Cranberry (2)	Urinal Intensiv	Walmark (Poland)	Tablet	*Vaccinium macrocarpon, Solidago virgaurea,* vitamin D	Urinary tract health, cystitis, diuretic, antispasmodic
Cranberry (2)	Urinal	Walmark (Poland)	Tablet	*Vaccinium macrocarpon, Solidago virgaurea,* vitamin D	Urinary tract health, cystitis, diuretic, antispasmodic

<div align="center">**Table 1.** *Cont.*</div>

Sample Type	Product Name	Manufacturing co.	Pharmaceutical Form	Composition	Indications
Cranberry (1)	High Strength Cranberry	Swisse (Australia)	Soft capsule	*Vaccinium macrocarpon*	Urinary tract health, cystitis, antioxidant
Cranberry (1)	Urell	Pharmatoka (France)	Capsule	*Vaccinium macrocarpon*	Urinary tract health, cystitis, antioxidant
Cranberry (2)	Monurelle	Zambon (Spain)	Tablet	*Vaccinium macrocarpon*, ascorbic acid	Urinary tract health, cystitis, antioxidant
Cranberry (2)	Arandano Rojo	Santiveri (Spain)	Tablet	*Vaccinium macrocarpon*, ascorbic acid, *Echinacea purpurea*	Urinary tract health, cystitis, antioxidant, immune system activation
Cranberry (2)	Antiox	NaturTierra (Spain)	Tablet	*Vaccinium macrocarpon*, ascorbic acid	Urinary tract health, cystitis, antioxidant
Raspberry, tea and others (3)	Cetonas de Frambuesa	Drasanvi (Spain)	Tablet	*Rubus idaeus, Mangillera Indica, Camellia sinensis, Paullina cupana, Euterpe olercarcea,* L-carnitine	Slimming, increasing fat metabolism, diuretic, stimulant,
Raspberry and tea (3)	Raspberry Ketones	Aquilea (Spain)	Tablet	*Rubus idaeus, Camellia sinensis,* pyridoxine hydrochloride	Slimming, increasing fat metabolism, diuretic, stimulant
Black grape (4)	Resverasor	Soria Natural (Spain)	Tablet	*Vitis vinifera* (skin and seeds)	Antioxidant, antiaging
Black grape (5) and others	Resveratrol	Aquilea (Spain)	Tablet	*Vitis vinifera* (seeds), *Allium cepa, Polygonum cuspidatum*	Antioxidant, antiaging
Black grape, (5) pomegranate	Revidox	Stillvid (Spain)	Capsule	*Vitis vinifera* (fruit extract), *Punica granatum*	Antioxidant, antiaging
Grapevine (5)	Arkocápsula Vid Roja	Arkopharma (Spain)	Capsule	*Vitis vinifera* (leaves extract)	Promoting blood circulation antioxidant,
Artichoke (6)	Alcachofa	Drasanvi (Spain)	Capsule	*Cynara scolymus*	Hypolipidemic, detox
Artichoke (6)	Alcachofa	Arkopharma (Spain)	Capsule	*Cynara scolymus*	Hypolipidemic, detox
Artichoke (6)	Alcachofa	Aquilea (Spain)	Capsule	*Cynara scolymus*	Hypolipidemic, detox
Artichoke (6)	Alcachofa	Roha (India)	Tablet	*Cynara scolymus*	Hypolipidemic, detox
Antiox mixture (7)	Antiox Forte	Santiveri (Spain)	Capsule	*Vitis vinifera, Rubus idaeus, Vaccinium corymbosum, Punica granatum, Fragaria vesca, Vaccinium macrocarpom, Vaccinium myrtillus*	Detox, antioxidant
Antiox mixture (8)	Venox	Drasanvi (Spain)	Capsule	*Vitis vinifera, Aesculus hippocastanum, Ruscus aculeatus, Hamamelis virginiana, Ribes nigrum, Vaccinium myrtillus, Ginkgo biloba,* ascorbic acid, vitamin A and E	Detox, antioxidant, promoting blood circulation

2.4. Sample Treatment

Samples were treated according to an extraction procedure described elsewhere [35]. Briefly, 10 capsules/tablets were randomly collected, crushed, mixed, and homogenized to obtain a representative laboratory sample. Then, 0.1 g of powder were treated with 5 mL of MeOH/H$_2$O/HCl (70:29:1 *v/v/v*) in 15-mL conical tubes. Analytes were recovered by UAE with the Branson 5510 ultrasonic bath (9.5-litre tank filled with water), working at a frequency of 40 kHz and power of 130 W for 30 min at a controlled temperature of 55 °C. Afterward, the extracts were centrifuged for 15 min at 3200× *g* and filtered through nylon membranes of 0.45 µm pore size (20 mm diameter, Macherey-Nagel, Düren, Germany). Extractions were carried out in triplicate. A quality control (QC) solution was prepared by

mixing 100 μL of each sample extract. The QCs were used to assess the repeatability of the chromatographic data and the quality of the PCA and PLS–DA models.

2.5. Chromatographic Method

The method consisted of precolumn derivatization of flavanols using DMAC reagent and further analysis by HPLC–UV/Vis. Derivatives were generated by mixing 20 μL DMAC solution with appropriate volumes of standards or samples and diluting up to 500 μL with H_2O/MeOH (75:25 *v/v*). The reaction was developed at 90 °C for 30 min, and the resulting mixture was injected into the HPLC–UV/Vis system. Compounds were separated by RP mode in a Kinetex C18 (100 mm length × 4.6 mm I.D, 2.6 μm particle size) with a C18 precolumn (4.00 mm length × 3.00 mm I.D) from Phenomenex (Torrance, CA, USA) using 0.1% (*v/v*) HCOOH and MeOH as the components of the mobile phase. The elution gradient was as follows: 3% to 95% MeOH, from 0 to 13 min (linear increase); 95% MeOH from 13 to 23 min; 95% to 3% MeOH, from 23 to 23.1 min (linear decrease). The column was conditioned with 3% MeOH for 5 min before the next run. The volume of injection was 5.0 μL, the flow rate was 0.4 mL min^{-1}, and the separation temperature 20 °C. The chromatogram was recorded at 640 nm for specific monitoring of flavanol derivatives.

2.6. Data Analysis

PLS-Toolbox (Eigenvector Research, Manson, WA, USA) working with MATLAB was used for the exploratory and classification studies of 21 different nutraceutical samples. Principal component analysis (PCA) and partial least square–discriminant analysis (PLS–DA) were used for sample characterization using both chromatographic fingerprints of samples of studied flavanols. Data were taken within the working range of 9.61 to 16.64 min. The plots of scores showing the distribution of the samples on the principal components (PCs) on PCA and the latent variables (LVs) on PLS–DA were used to differentiate the samples according to their fruit variety. The plot of loadings allowed identifying the most discriminant species.

The robustness, significance, and repeatability of chromatographic fingerprints were assessed from the study of the quality controls (QCs) which, in the scores' plots of PCA, should be located close to the center of the model in a compact cluster, meaning that all of them are similar in terms of intensities and retention times.

3. Results and Discussion

3.1. Optimization of Chromatographic Fingerprints

The separation of derivatized flavanols using DMAC reagent resulted in a challenging issue due to the structural similarity of derivatives and occurrence of side products. Preliminary studies were carried out using a mixture of standards, consisting of catechin, epicatechin, epigallocatechin gallate, and procyanidins A_2, B_2, and C_1 at 20 mg L^{-1} each. Flavanols were derivatized at 90 °C for 30 min according to Vidal et al. [45] and afterward injected into the chromatograph. It was found that procyanidin B_2 and C_1 derivatives underwent degradations, thus producing a complex mixture of products. Moreover, studies with pure standards indicated that the separation of catechin and epicatechin derivatives was not achieved.

The following research was focused on the separation of flavanol derivatives from sample extracts. The principal criterion of the optimization was to achieve the richest compositional profiles for the nutraceutical samples under study in a minimum running time to be further used as a source of information for descriptive purposes. An experimental plan was designed to assay the influence of methanol percentage and gradient time on the separation. Levels selected were 3, 5, and 10% (*v/v*) for the initial methanol and 8, 13, and 20 min for the gradient time. In this method, the optimal separation was mathematically assessed from the number of peaks (n$_{peaks}$) detected in the chromatograms and the retention time (t$_R$) of the last peak: D = (dn$_{peaks}$ × dt$_R$)$^{1/2}$, with dn$_{peaks}$ and dt$_R$ being the normalized values of the number of peaks and retention time, respectively.

Figure 2 shows the effect of initial methanol percentage and gradient time on the separation of derivatized flavanols expressed as a desirability function D and the representative chromatograms under selected conditions.
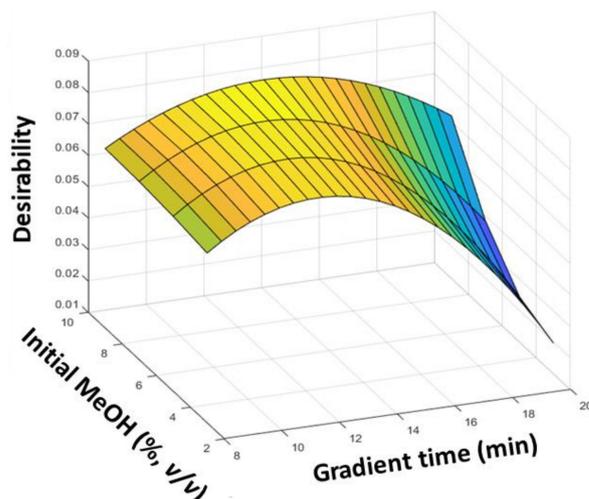

Figure 2. Optimization of separation of flavanol derivatives by HPLC–UV/Vis with DMAC precolumn derivatization: Response surface plot with the influence of the initial MeOH percentage and gradient time on the separation performance expressed as a desirability function.

The results depicted in Figure 2 indicated that the best separation was achieved with 10% initial methanol and 13 min gradient time; hence, they were selected for further studies. As can be observed in Figure 3, the chromatograms of sample and standards displayed various strongly overlapping peaks, which were attributed to unknown flavanol derivatives and degradation products of procyanidins. For instance, the procyanidin C_1 trimer undergoes a remarkable degradation because subunits are only linked by single B-type bonds. In contrast, molecules with both B- and A-type bonds linking their units, such as procyanidin A_2, were much more stable. In conclusion, despite the great efforts to optimize the separation of derivatives, both the structural similarity of the derivatives and the occurrence of degradations hindered obtaining fully resolved peaks. Compounds in the chromatograms were identified from the corresponding standards, but eventually, this method was not recommendable for quantitative purposes. Regardless, it should be emphasized that the identification or quantification of compounds when working under fingerprinting approaches was no longer necessary and, in consequence, the potential interest of the resulting sample fingerprints for characterization issues should not be underestimated. In the following section, an example of the descriptive performance of this fingerprinting approach is provided.
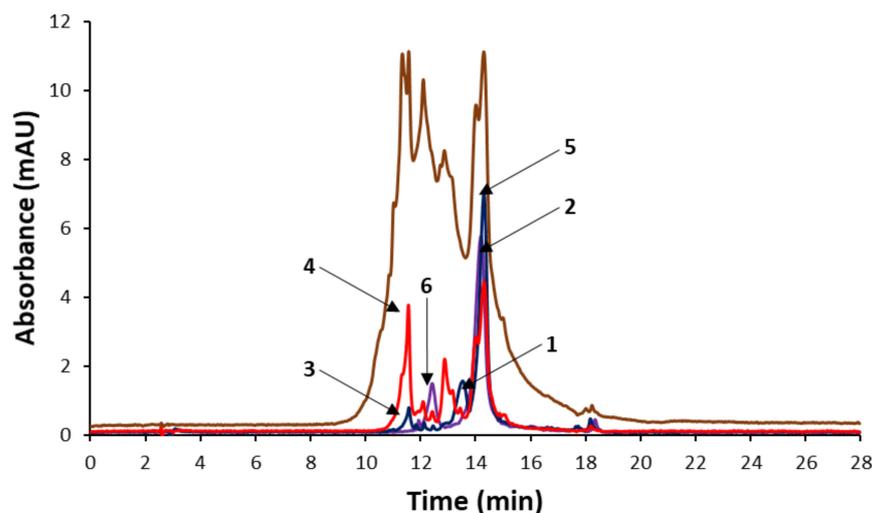
Figure 3. Chromatograms of the standards mixture and a cranberry sample under selected conditions as follows: 10% initial MeOH percentage; 13 min gradient time; UV/Vis detection at 640 nm. Compound assignment: 1 = epigallocatechin gallate; 2 = catechin; 3 = procyanidin B2; 4 = procyanidin C1; 5 = epicatechin; 6 = procyanidin A2.

3.2. Sample Characterization by PCA and PLS–DA

As can be observed in Figure 4, fingerprints from the HPLC–UV/Vis method with DMAC derivatization were highly selective for flavanol compounds. Unfortunately, as mentioned above, chromatograms were complex, with multiple unresolved peaks, because of the coelution of some derivatives (e.g., procyanidin C_1/B_2 and catechin/epicatechin) and the occurrence of degradation products.
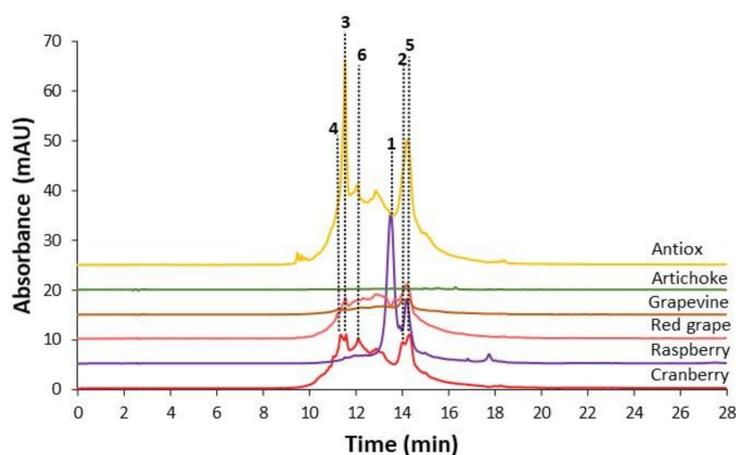

Figure 4. Representative chromatograms of various nutraceutical samples obtained by DMAC precolumn derivatization with UV/Vis detection at 640 nm. Sample assignation: yellow = antioxidant mixture; green = artichoke; brown = grapevine; pink = red grape; purple = raspberry and red = cranberry. Compound assignation from standards: 1 = epigallocatechin gallate; 2 = catechin; 3 = procyanidin B2; 4 = procyanidin C1; 5 = epicatechin; 6 = procyanidin A2.

It was found the composition of nutraceuticals differed in terms of concentration and distribution of flavanols depending on the extract ingredients. In general, catechin and epicatechin were the most abundant compounds in different types of samples, except on raspberry-based samples, where epigallocatechin gallate was the principal compound. Moreover, procyanidin A_2 was highly specific to cranberry. Grape and antiox mixtures of other antioxidant extracts were rich in procyanidin B_2 and C_1. Finally, artichoke samples displayed the lowest contents of flavanols.

For a comprehensive assessment of the sample behavior, chromatographic fingerprints were submitted to chemometric analysis. Preliminary PCA models recommended to average the replicates of each given sample to achieve more robust descriptive models. In this way, chromatographic drifts and intensity variations were minimized so that the processed data displayed better descriptive ability for discrimination between the fruit varieties. PCA and PLS–DA were applied to characterize and authenticate nutraceutical samples according to their plant ingredients. In the two cases, QCs were in a compact cluster, thus suggesting the robustness of the descriptive models. Samples manufactured from cranberry and other vegetables such as artichoke, raspberry, grape, and grapevine were compared to try to identify characteristic patterns depending on the flavanol composition. The results from PLS–DA depicted in Figure 5 showed some sample clustering such as for artichoke and cranberry, raspberry, and grape-based products. Moreover, samples combining various plant extracts appeared in positions close to those of the main ingredients. Finally, PLS–DA model allowed better differentiation between classes than the PCA model.
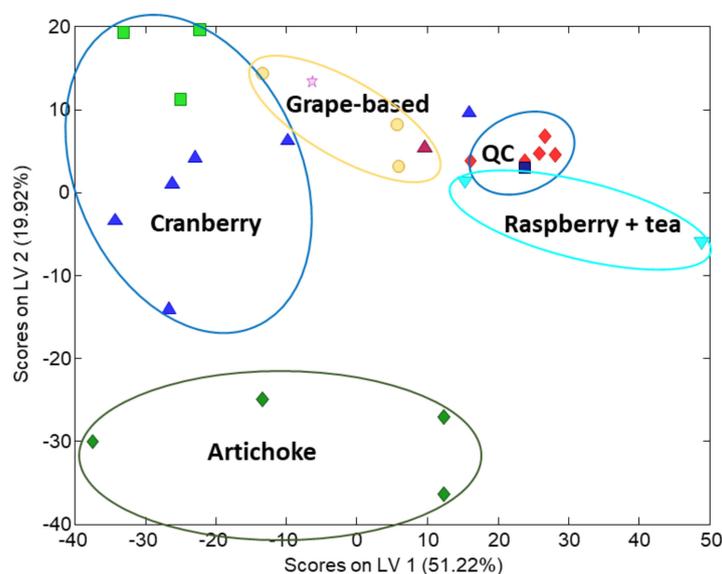

Figure 5. Characterization of nutraceuticals and dietary supplements by PLS–DA using the chromatographic fingerprints in the time range 9.61 to 16.64 min as the data. Scatter plot of scores of LV1 vs. LV2. Sample assignation: green square = cranberry (class 1); blue triangle (vertex up) = cranberry with others (class 2); triangle (vertex down) = raspberry + tea (class 3); star = black grape (seeds, class 4); circle = black grape (peel) and grapevine (class 5); green rhombus = artichoke (class 6); blue square = mixture of antioxidants (class 7); purple triangle (vertex up) = mixture of antioxidant (class 8); red rhombus = QCs.

To the best of our knowledge, DMAC has not been previously used in precolumn derivatization for addressing profiling or fingerprinting methods for the characterization of nutraceutical products. As described elsewhere, the assessment of the antioxidant power, which in this range of products is closely related to overall flavanol content, was often estimated from several spectrophotometric methods such as Folin–Ciocalteu, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), etc. [46–48]. For a more specific determination of a global flavanol index, DMAC spectrophotometric method was commonly employed [16,19–21,45]. In some cases, the vanillin method was used as an alternative to DMAC for the spectrophotometric determination of flavanols, such as in the quantification of catechins in *Malus silvestris* extracts [49]. Regardless, spectroscopic indexes only provided a rough estimation of global contents, meaning that other experimental approaches should be used for deeper insights on the composition of the different flavanol molecules, especially those based on liquid chromatography and high resolution spectrometric techniques.

Chromatographic methods working with underivatized catechins seem to be a good option to gain more detailed information on monomeric and oligomeric flavanols. In this regard, several studies were published in which concentrations of flavanols were exploited as a source of analytical information of the characterization of medicinal extracts such as berry-based products [25,44]. Weseler and Bast reviewed the relevance of grape seed extracts as a rich source of flavanols and other phenolic compounds with indications in the treatment of vascular and inflammatory alterations [50]. The authors pointed out the importance of the characterization and standardization of the composition of plant-derived extracts, which is essential to achieve the desired bioactivity and to establish a cause–effect relationship between the intake of that product and its health effect. In the paper, the principal analytical strategies to deal with this characterization were covered, including colorimetric assays, liquid chromatography (LC–UV, LC–MS), and nuclear magnetic resonance. In another example, Paschoalinotto et al. evaluated various health-promoting tisanes exhibiting bioactive properties (e.g., antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and antidiabetic activities) on the basis of flavanol and hydroxycinnamic acid profiles obtained by liquid chromatography [51]. Data treatment by PCA allowed the most promising tisane to be selected according to the activity. In the publication by Jia et al., a high-throughput method for the determination of a wide range of flavonoids in antioxidant nutraceuticals was developed using liquid chromatography coupled to mass spectrometry (LC–MS) as the analytical technique [52]. In another similar case, citrus-based products with potential nutraceutical applications were characterized by LC–MS [53]. Another example relied on phytochemical fingerprints by liquid chromatography and antioxidant indexes to evaluate the remarkable health-promoting value of *Prunus padus* extracts as a potential source of natural nutraceutical products [54].

Overall, chemical methods are commonly used to try to assess the bioactivity of nutraceuticals in a fast, efficient, and robust way. Some studies apply global spectroscopic indexes although chromatographic methods are more interesting for an individualized determination of specific target compounds or even for the generation of sample fingerprints with great possibilities in characterization and authentication. In this regard, our proposal combining DMAC labeling and chromatographic separation of derivatives results in an attractive methodology for the study of nutraceuticals.

4. Conclusions

The possibilities of flavanols as descriptors of plant-based extracts were here demonstrated from a comparison of the behavior of nutraceuticals made with extracts of cranberry, artichoke, raspberry, grape, etc. The new methodology relied on precolumn derivatization of flavanols with DMAC and analysis of the resulting derivatives with HPLC–UV/Vis. Chromatograms recorded at 640 nm were suitable to generate complex fingerprints dealing with flavanol composition, but their quantitative performance was limited because of degradation issues. The exploratory study of fingerprints by principal component analysis (PCA) and partial least squares–discriminant analysis (PLS–DA) allowed nutraceuticals to be discriminated according to their main plant ingredients. Cranberry samples were the richest and artichoke samples the poorest in terms of flavanol contents. Although the method was here applied to some selected types of samples, it can be extended to a broader range of nutraceuticals and food supplements.

In future studies, the HPLC–UV/Vis method with DMAC precolumn derivatization will be investigated in more detail to assess the degradation of flavanol derivatives and thus obtain better chromatographic separation and characterization of nutraceutical samples.

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