Methods and Applications in Fluorescence

Application of multidimensional and conventional fluorescence techniques for classification of beverages originating from various berry fruit

To cite this article before publication: Ewa Sikorska *et al* 2019 *Methods Appl. Fluoresc.* in press https://doi.org/10.1088/2050-6120/ab6367

Application of multidimensional and conventional fluorescence techniques for classification of beverages originating from various berry fruit

Ewa Sikorska[1*], Katarzyna Włodarska[1], and Igor Khmelinskii[2]

[1]Institute of Quality Science, Poznań University of Economics and Business, al. Niepodległości 10, 61-875 Poznań, Poland; Fax: +48 61 8543993, Tel: +48 61 8569040, e-mail: Ewa Sikorska (ewa.sikorska@ue.poznan.pl); Katarzyna Włodarska (katarzyna.wlodarska@ue.poznan.pl)

[2]Universidade do Algarve, FCT, DQF and CEOT, Campus de Gambelas, Faro 8005-139, Portugal, e-mail: Igor Khmelinskii (ikhmelin@ualg.pt)

Running title: **FLUORESCENCE FOR CLASSIFICATION OF BERRY BEVERAGES**

Corresponding author:

Ewa Sikorska

Faculty of Commodity Science

Poznań University of Economics and Business

al. Niepodległości 10

61-875 Poznań

Poland

*ewa.sikorska@ue.poznan.pl

fax: +48 61 8543993, tel: +48 61 8569040

Abstract

The objectives of this study were to characterize fluorescence of beverages from berry fruit, including chokeberry, blackcurrant, raspberry and strawberry, and to develop classification models based on different types of fluorescence spectra to identify beverages depending on the fruit species. Total fluorescence spectra (excitation-emission matrices, EEMs) and total synchronous fluorescence spectra (TSFS) were recorded for a series of commercial berry fruit beverages. An analysis of EEMs using parallel factor analysis (PARAFAC) revealed four components characterized by the excitation/emission maxima at 275/326, 319/410, 414/600, and 360/460 nm, respectively. Based on the spectral profiles, these components were assigned to various groups of phenolic compounds. Partial least squares discriminant analysis was used to develop the classification models. The analysis was performed on PARAFAC scores, the unfolded EEMs (uEEMs), unfolded TSFS (uTSFS), and additionally on conventional emission spectra (EMS) measured at particular excitation wavelengths and single synchronous fluorescence spectra (SFS). The classification models with the same average classification error of 4.86% were obtained for the analysis of both the entire uEEMs and uTSFS. Among models based on the individual spectra, the lowest error of 4.42% was obtained for SFS measured at $\Delta\lambda$=40 nm, and an error of 7.64% was obtained for EMS measured at the excitation wavelength of 360 nm. The classification model based on the PARAFAC scores had the highest error of 15.27%. The present results show good potential of fluorescence as rapid and reagent-free tool for authenticity evaluation of berry beverages.

Keywords: Berry fruit beverages; Excitation-emission matrix; Synchronous fluorescence; PARAFAC; PLS-DA; Classification

Introduction

Over the past years the application of spectroscopic techniques has gained increasing attention in food analysis [1]. The spectra measured using various techniques provide chemical fingerprints for particular food samples. The unique spectral pattern of a food product depends on the chemical components present, their interactions, and may be also affected by the physical properties of the sample. The main advantage of the spectroscopic techniques is that the analytical information provided by the respective spectra may be obtained by relatively easy and non-invasive measurements directly on the food samples. The use of chemometric methods in the analysis of spectral data is necessary due to the limited selectivity of signals caused by overlapping spectral bands of different food constituents. The main objectives of using chemometric methods are to identify patterns in the data, classify the samples, and model the relationships between the spectra and the evaluated properties.

Spectroscopic techniques coupled to chemometrics provide an alternative to conventional methods in high-throughput determinations of properties of foods, including fruit and fruit-based products [1]. The method most intensively used in the food analysis is the near-infrared spectroscopy, which nowadays is one of the basic tools in the routine food analysis and process control. The feasibility of other spectroscopic techniques, including mid infrared, ultraviolet-visible, Raman, nuclear magnetic resonance and fluorescence, has also been demonstrated in many studies.

A growing number of studies show that electronic spectroscopy may be a valuable alternative to vibrational spectroscopic techniques for studying foods. In particular, fluorescence spectroscopy coupled with multivariate analysis has been successfully used as fingerprinting techniques in food quality evaluation. In addition to the advantages common to all of the spectroscopic techniques, fluorescence is more selective and sensitive than absorption

spectroscopy and is inherently multidimensional, providing more comprehensive information [2].

Conventionally the sample fluorescence is characterized by the emission and excitation spectra, which represent the emission intensity as function of the wavelength of the emitted radiation, measured at constant wavelength of excitation or emission, respectively. However, food samples usually contain several important fluorophores, thus the measurements of conventional emission or excitation spectra at a selected excitation or emission wavelength are not sufficient to characterize all of these fluorophores. A more comprehensive characterisation of multifluorophoric systems is obtained by synchronous fluorescence spectroscopy, which represents fluorescence intensity as a function of the simultaneously scanned emission and excitation wavelengths, usually with a constant offset between the two ($\Delta\lambda = \lambda_{em} - \lambda_{exc}$) [3]. The profile of a synchronous fluorescence spectrum is thus dependent on the $\Delta\lambda$ value. The synchronous fluorescence spectra (SFS) in comparison with the emission spectra are characterized by higher selectivity and sensitivity, reduced overlapping of the spectral bands from different analytes due to the narrowing of their spectral widths, and reduction of the unwanted contribution of the scattered light [4].

The most comprehensive characterization of multifluorophoric systems is obtained using multidimensional techniques such as the measurements of total fluorescence spectra (TFS), also known as an excitation-emission matrices (EEMs), and the measurements of total synchronous fluorescence spectra (TSFS). The excitation-emission matrix (EEM) is obtained by recording emission spectra for a series of excitation wavelengths, thus providing comprehensive characterization of the absorption and fluorescent properties of all of the emitting components in the sample tested [2]. The total synchronous fluorescence spectrum is obtained by recording the SFS over the range of $\Delta\lambda$ values [5].

To fully utilize the analytical potential of the unique features of fluorescence, appropriate chemometric methods are used to analyze the recorded spectral matrices.

A considerable number of minor and trace components of beverages, which belong to different chemical classes, exhibits detectable fluorescence [2]. Food-relevant fluorescent compounds include aromatic amino acids, both as individual compounds or present in proteins, some vitamins, chlorophyll and its derivatives, process-derived compounds, and some food additives and contaminants. Among these, phenolic compounds are an important group of natural fluorophores present in beverages of plant origin. Due to the variety of their structures, these compounds exhibit different properties, and many of them are fluorescent. Fluorescence has been successfully used to evaluate different aspects of the quality of various food products, including liquid phenolic-containing products, such as wine, spirit drinks, fruit juices, olive oil, coffee, and tea [6].

An important group of beverages with high contents of phenolic compounds is the one produced from berry fruit. These fruit have attracted in recent years an increasing attention due to their nutritional quality and delicious and unique flavor [7]. The term “berry” in the pomological nomenclature refers to a diverse group of edible fruit of small size, round, and usually juicy, characterized by an intense color ranging from red to purple and blue, and taste from sweet to sour or bitter. This group of fruit is also called “red fruit” or “soft fruit” [8]. Not all fruit classified as berries in the pomological sense are true berries according to the botanical definition [9].

Berries are a good source of macro- and micronutrients [10]. They contain high amounts of dietary fiber (cellulose, hemicellulose, and pectin), vitamins A, C, and E, vitamins of the B group, and some of the essential micronutrients [8]. Phenolic compounds are an important bioactive component in berries [7], responsible for the high antioxidative capacity, and due to perceived effect of berry consumption on the prevention of chronic diseases [11]. Berry fruits

are consumed in fresh and processed form [8, 12]. In addition to raw fruit, consumption of the berry beverages may be an important element of a healthy diet. Popular beverages are obtained, among others, from chokeberry, blackcurrant, strawberry and raspberry.

Authenticity of fruit juices is one of the important aspects of their quality. Fraudulent practices in the beverage industry include mislabelling of product species and their geographical origin, dilution with water, and replacement of expensive ingredients with cheaper substitutes.

The profiles of bioactive compounds in berries are strongly affected by the genotype of fruit – species and variety within the species [10], and thus have been used in authenticity studies. For example, the anthocyanin profiles have been used for taxonomy of berry fruit, and also to determine the authenticity of berry-derived food products [11]. Advanced analytical methods that were used for authenticity evaluation of berry fruit juices include polyphenolic profiling using HPLC [13], liquid chromatography quadrupole time-of-flight mass spectrometry [14], UHPLC-HRMS (Orbitrap) [15] and DNA barcoding method [16]. Non-targeted fluorescence fingerprinting analysis may be a valuable alternative to the conventional and chemical profiling methods [1]. So far, fluorescence has been successfully applied for authenticity testing of various beverages including wine [17-22], ice cider [23], spirit drinks [24, 25], apple juice [26-28], orange juice [29], coffee [30], and tea [31-33].

The aim of the present paper was to explore the fluorescence of commercial berry beverages, obtained from chokeberry, blackcurrant, strawberry and raspberry, and to test its usage for the classification of products originated from different fruit. Different techniques of fluorescence measurements, including multidimensional total fluorescence spectra and total synchronous fluorescence spectra, and synchronous fluorescence spectra and emission spectra were explored and compared.

2. Material and methods

2.1. Berry beverage samples

The studied sample set consisted of juices, nectars and syrups produced from blackcurrant (*Ribes nigrum*), chokeberry (*Aronia melanocarpa*), strawberry (*Fragaria* × *ananassa*) and raspberry (*Rubus idaeus*). A total of 48 berry products that were available on the Polish market were evaluated in this study. The studied products included 12 chokeberry beverages: juices (8), nectars (1), and syrups (3); 12 blackcurrant beverages: juices (5), nectars (6), and syrups (1); 12 raspberry beverages: juices (8), and syrups (4); and 12 strawberry beverages: juices (5), nectars (1), and syrups (6).

2.2. Fluorescence measurements

The fluorescence spectra were recorded using a Fluorolog 3-11 spectrofluorometer (Spex-Jobin Yvon, France). The total fluorescence spectra (excitation-emission matrices, EEMs) were obtained by recording the emission spectra in the 300-650 nm range with the excitation in the 270-500 nm range, at 5 nm steps in the excitation wavelength. The TSFS were acquired by recording the synchronous spectra in the 250-600 nm excitation range with the emission-excitation offsets ($\Delta\lambda$) in the 10-200 nm range, with a 10 nm step. The individual synchronous fluorescence spectra present the fluorescence intensity as a function of the excitation wavelength. The emission and synchronous fluorescence spectra were corrected for the wavelength-dependent response of the system.

The excitation and emission slit widths were 3 nm. The acquisition interval and the integration time were maintained at 1 nm and 0.1 s, respectively.

The undiluted samples were measured directly in a 10 mm fused-silica cuvette applying front face geometry. To reduce the scattered light effects, the samples were centrifuged before measurements (14000 rpm for 5 min).

2.3. Data analysis

Data arrangement

The EEMs were arranged for the numerical analysis into three-way array with the size of 48 × 360 × 47 elements (number of samples × number of emission wavelengths × number of excitation wavelengths) or held in the unfolded array with the dimensions of 48 × 16 920 elements, given by number of samples × (number of emission wavelength *multiplied by* number of excitation wavelengths). The three-way EEMs were unfolded along the sample mode (Supplementary material, Figure S1). Additionally, individual emission spectra measured at the particular excitation wavelength in the range of 270–500 nm with 10 nm step were analyzed.

The recorded TSFS were held in an array with the size of 48 × 20 × 351 elements (number of samples × number of excitation wavelengths × number of wavelength offsets). The array was unfolded for numerical analysis along the sample mode, forming a matrix with the dimensions of 48 × 7020 elements (number of samples × number of excitation wavelengths *multiplied by* number of wavelength offsets) (Supplementary material, Figure S2). Additionally, individual synchronous fluorescence spectra measured at the particular wavelength offsets ($\Delta\lambda$) in the range of 10–200 nm with 10 nm step were analyzed.

Parallel factor analysis (PARAFAC)

Parallel factor analysis (PARAFAC) was used to decompose the EEMs into the contributions of the individual fluorescent components [34], (please consult Supplementary material for

more details about PARAFAC method). Three-way data EEMs array was used in the PARAFAC analysis. The Rayleigh scattering contributions to the EEMs were removed by inserting the interpolated values. Non-negativity constraints were applied to the excitation and emission spectra and the concentrations. The optimal number of components in the PARAFAC models was chosen based on the explained variance, core consistency diagnostic (CORCONDIA) and split-half analysis.

Partial least squares discriminant analysis (PLS-DA)

The PLS-DA was used for the development of classification models for the four classes of products originated from different fruit, based on fluorescence data [35] (please consult Supplementary material for more details about PLS-DA method). The separate PLS-DA models were developed using PARAFAC scores as the **X** matrix, the entire uEEMs, the entire uTSFS, individual emission spectra, and individual synchronous fluorescence spectra. The response matrix (**Y**) in the PLS-DA analysis was a dummy matrix with four columns containing class membership information for each of the samples. In particular, the respective variable was set to 1 for all of the juices originating from a particular fruit and to 0 for the other juices.

All models were developed for mean-centered data. Additionally, unit vector normalization was applied at the model optimization step.

Cross-validation was used to assess the optimal number of components and to estimate the model performance. This procedure is based on selection of different subsets of the samples, which are used for model building (training set) and testing (test set). The steps of model building and testing are repeated several times with different samples subsets, and the same samples may be used in the training and test sets in different runs. The Venetian-blinds

variant of cross-validation with 10 data splits was applied, in which every 10^{th} sample was selected for test set, starting from the first sample to the last.

The optimal number of components was selected as the minimum in the plot of the average classification error rate as a function of the number of components. The performance of models was estimated on the basis of the classification error rate, sensitivity and selectivity for individual classes, and the average classification error rate [35]. The sensitivity of a particular class was defined as the fraction of the samples that were correctly identified as the members of that class. The specificity of a particular class was defined as the fraction of samples of other classes that were correctly rejected by the model. The classification error rate for a particular class was calculated as the fraction of samples that were classified incorrectly. The average classification error rate was calculated as the mean value of classification error rates for the four classes studied. All of these parameters were expressed in percentages.

The Variable Importance in Projection (VIP) was used to identify variables that significantly contribute to the PLS-DA models [36]. VIP provides a measure of explanation of the variance of **X** by each of the variables and, simultaneously, of the correlation of **X** with **Y**.

The data analysis was performed using Solo v. 5.0.1 software (Eigenvector Research Inc., USA).

3. Results and discussion

3.1. Fluorescence characteristics of berry juices

Total fluorescence spectra, excitation-emission matrices

The TFS (or EEMs) of all of the beverages studied were obtained by recording the emission spectrum for a series of excitation wavelengths, thus they present the fluorescence intensity as function of both excitation and emission wavelengths. Figure 1 shows the EEMs of representative samples in each of the four studied categories of beverages. Similar features are

present in all of the recorded spectra. Specifically, three emission bands are observed, with their respective excitation/emission maxima at (I) 276-280/314-338 nm, (II) 310-345/390-455 nm, and (III) 380-465/585-645 nm. The differences in the exact positions of the maxima and the relative intensities of the particular bands are observed for particular juices. Moreover, a fourth (IV) emission band is present with the excitation/emission maxima at 386-420/499-560 nm in some of the strawberry beverages only.

Insert Figure 1

Total synchronous fluorescence spectra

TSFS of the beverages studied were measured by recording the synchronous fluorescence spectra for the range of $\Delta\lambda$ values. The synchronous measurements rely on simultaneous scanning of the excitation and emission wavelengths with a constant offset $\Delta\lambda$ between them. The single SFS usually presents the fluorescence intensity as function of the excitation wavelength. Thus, the TSFS present the fluorescence intensity as a function of the excitation wavelength and the $\Delta\lambda$ offset. Figure 2 illustrates the overall characteristics of the TSFS, of the four representative samples in each of the beverage categories studied.

Insert Figure 2

The TSFS show the narrowing of the bands and the shift of their maxima to shorter wavelengths compared to the EEMs. The TSFS of all of the beverages studied, similarly to EEMs, show some common patterns. Three distinct emission zones are present, with varied exact positions of the maxima and their relative intensity for different beverages. These bands in TSFS correspond to the respective bands in the TFS. The maxima of these spectral bands are observed in the following $\Delta\lambda/\lambda_{exc}$ ranges: (I) 43-72/270-282 nm, (II) 66-138/315-351 nm and (III) 150-190/410-480 nm**.** An additional emission band (IV) is observed in strawberry beverages, with the respective maxima at $\Delta\lambda/\lambda_{exc}$ 98-137/360-409 nm.

3.2. Exploratory analysis of fluorescence spectra

Parallel factor analysis of total fluorescence spectra

A detailed insight into the EEMs patterns of the beverages studied was obtained by the PARAFAC analysis. The EEMs fulfill the trilinearity conditions; every fluorophore has unique excitation and emission spectral profiles independent of the changes in the other two modes, thus PARAFAC may be used for their analysis. The objective of this analysis was to resolve the EEMs into the contributions of the individual fluorophores.

Based on the value of explained variance (97.9%), core consistency value (46) and analysis of both the residuals and the loadings, an optimal PARAFAC model for all beverages studied was identified as having four components. The excitation and emission loadings of these four fluorescent components and their respective relative contributions are presented in Figure 3.

Insert Figure 3

These PARAFAC components had their maxima at the following excitation/emission wavelength pairs: 275/326 nm (component 1), 319/410 nm (component 2), 414/600 nm (component 3), and 360/460 nm (component 4). A tentative assignment of the PARAFAC components is based on the literature data. The native fluorescence of berry beverages may originate from several groups of chemical compounds; phenolic compounds being an important group. The phenolics present in berry product include anthocyanins, phenolic acids, tannins, and flavonoids. Berries also contain vitamins A, E, and the B group vitamins, which are all fluorescent [10].

The first PARAFAC component with its excitation/emission maxima at 275/326 nm may be ascribed to hydroxybenzoic acids and catechins. The fluorescence of hydroxybenzoic acids found in red fruit juices (gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, and syringic acid) were reported in the excitation maximum range of 260-290

nm and the emission maximum range of 340-360 nm [13]. The emission of catechin and epicatechin was reported at 280 nm in excitation and at 325 nm in emission [13].

The second component with the excitation/emission maxima at 319/410 nm may correspond to hydroxycinnamic acids that show fluorescence with the excitation maximum of 310-340 nm and the emission maximum ranging from 420 to 455 nm [13]. The major hydroxycinnamic acids found in berries are ferulic, caffeic and p-coumaric acids and caffeoylquinic esters [11]. Blackcurrant has high contents of p-coumaric acid and caffeic acid [12]. Ellagic acid that is the dominant acid in strawberries and raspberries shows absorption maxima at 253 and 366 nm, and the fluorescence maximum at 425 nm. It is present in either the free form or esterified to glucose in hydrolysable ellagitannins [12].

The third component had its excitation/emission maxima at 414/600 nm and may be tentatively ascribed to anthocyanins. Berries are particularly rich in anthocyanins, which are responsible for their characteristic colors [11]. The anthocyanin composition of berries depends on the species and varieties [10]. The dominant anthocyanins in the four studied fruit are: delphinidin-3-rutinoside – in blackcurrant, cyanidin-3-galactoside – in chokeberry, cyanidin-3-sophoroside – in raspberry, and pelargonidin-3-glucoside – in strawberry. Anthocyanins are weakly fluorescent in solution, however, aggregation or complexation to other molecules can induce significant fluorescence of the resulting anthocyanin-derived complexes [37]. The orange to red fluorescence of anthocyanins was reported in the emission wavelength range from 595 to 630 nm [38].

The forth PARAFAC component exhibited an excitation spectrum with its maximum at 360 nm and emission with its maximum at 460 nm. This fluorescence may originate in quercetin and kaempferol, flavonols that are particularly abundant in berry fruit [10]. Kaempferol fluorescence was reported at the excitation/emission maxima of 365/445-450 nm [38]. The emission maximum for quercetin was reported at 400-420 nm, with the excitation at 260-262

nm [18] or at 480 nm with the excitation at 427 nm in tartrate buffer (pH=7) and 13% ethanol [19]. Another study found that quercetin fluorescence was pH-dependent, with dual emissions observed in aqueous solutions (pH=5), with the maxima at 455 nm and 521 nm, attributed to the normal and the tautomeric form, respectively [39].

According to Sádecká et al. [40] the PARAFAC component observed in brandy with the maxima at 390/482 nm in excitation and emission was ascribed to coumarins, tannins, phenols and flavonols. Note that both hydrolysable and condensed tannins are found in berry fruit. Therefore, it rather seems that hydrolysable tannins should contribute more to the forth PARAFAC component. These compounds are derivatives of gallic and ellagic acids that have been found in strawberries and raspberries, and are less common in other berry fruit [12]. The fluorescence maximum of tannins was reported at 500 nm, with the excitation rage of 360-380 nm [38].

Condensed tannins are oligomers or polymers, usually of catechin and epicatechin [11]. In berry fruit, the largest quantity of condensed tannins with a high degree of polymerization is found in chokeberry [12].

Figures 3c and 3d show the contributions of each of the four PARAFAC components to the EEMs of the individual juices. The great variability of spectral properties within particular classes of beverages originating from the same fruit is observed. At the same time, there are some differences between different classes. The chokeberry and blackcurrant juices had generally lower contribution of component 1 as compared to the raspberry products. At the same time, strawberry beverages show an intermediate contribution of this component. All of the juices presented a similar contribution of the component 2. The chokeberry, blackcurrant, and raspberry juices were characterized by a similar contribution of the component 3, while the strawberry juice had the highest contribution of that component. Some chokeberry products had very low or zero contribution of component 4, blackcurrant and raspberry

showed low - to - intermediate contribution while strawberry products had the highest contribution of that component. The PARAFAC scores provided some discrimination among the juices according to their origin. Some discrimination of beverages was observed in the planes defined by the first and the second, and the third and the fourth components. The strawberry beverages were discriminated from the other three groups of juices in the plane that was defined by the third and the fourth components.

3.3. Multivariate classification models

The PLS-DA method was applied for discriminating the beverage samples into the four categories. The analyses were performed separately on the PARAFAC scores, on the entire uEEMs, entire uTSFS, and on the individual SFS and EMS. Raw and normalized spectral data were analysed. The characteristics of the resulting classification models are presented in Table 1.

Insert Table 1

PARAFAC-PLS-DA

The PLS-DA model based on the PARAFAC scores was characterized by the relatively high classification error of 15.27%. The errors for the individual classes ranged from 6.94% for chokeberry to 27.77% for blackcurrant. The highest sensitivity and specificity were thus obtained for chokeberry and strawberry beverages.

uEEMs-PLS-DA

The PLS-DA analysis of uEEMs led to the considerably better classification results. The average classification error was 4.86%. Perfect classification was obtained for the strawberry

beverages. The other classes were classified with similar error values of 6.94% for the chokeberry and raspberry beverages and 5.55% for blackcurrant.

The Variable Importance in Projection (VIP) was used to identify the spectral ranges that significantly contribute to the discrimination between the classes of beverages.

Insert Figure 4

Figures 4 shows the respective VIP plots for each of the classes studied. The VIP provides a measure of the significance of variables in a discrimination model. The variables that are characterized by the VIP values higher than unity, contribute significantly to the discrimination between the classes studied. The analysis of the respective VIP plots revealed that the emission spectra measured at lower excitation wavelengths contribute significantly to the discrimination of all of the classes. For chokeberry and strawberry the contribution of the emission spectra measured in the excitation wavelength range of about 300-380 nm is also important.

uTSFS-PLS-DA

In the next step the uTSFS were analysed. The average classification error for this model was the same as that for the uEEMs-PLS-DA model, Table 1. However, the classification performance for individual classes was different. The best classification results were obtained for chokeberry and strawberry beverages, while higher errors were obtained for blackcurrant and raspberry classes.

Insert Figure 5

Figure 5 shows the respective VIP plots for the uTSFS-PLS-DA model for each of the classes studied. Several spectral bands contribute to the discrimination of particular classes. A significant contribution of SFS in the $\Delta\lambda$ range of 20 to 40 nm was observed for all of the

classes studied. Moreover, SFS for Δλ between 60 and 100 nm also contribute significantly to the classification.

Individual EMS-PLS-DA

To test the usability of conventional emission spectra in beverage discrimination, PLS-DA models were developed using the individual emission spectra measured at the excitation wavelength range from 270 to 500 nm. The spectral data recorded every 10 nm were all tested. The models with the lowest average classification errors were obtained in the analysis of the normalized spectra. The main characteristics (classification errors and the number of latent variables) for the PLS-DA model are shown in Figure 6A for the individual normalized emission spectra.

Insert Figure 6

The classification performance of the tested PLS-DA models depended on the analyzed emission spectra. Generally, the models with lower classification errors were obtained for the spectra recorded as the lower excitation wavelengths. The classification errors below 10% were obtained for the emission spectra measured at the excitation wavelengths of 360, 340, and 290 nm. The model for the emission spectra recorded at the excitation wavelength of 360 nm had the best performance. This model was characterized by the average classification error of 7.64%, Table 1. Two classes – chokeberry and blackcurrant beverages – were classified with the same low error value of 2.8%; while the two other classes – raspberry and strawberry – with the relatively high error of 12.5%.

Individual SFS-PLS-DA

A series of PLS-DA models were developed for the SFS measured for Δλ from 10 to 200 nm with 10 nm step to test the potential of the individual SFS for the beverage classification. The

characteristics of the models for the raw SFS are presented in Figure 6B. The lowest average classification errors were obtained for the SFS measured at the $\Delta\lambda$ values below 40 nm. The best classification results were obtained for the SFS obtained at $\Delta\lambda = 40$ nm. The average error rate for this model was a little lower than that for the model based on the entire uTSFS. Among the individual classes, the best classification results were obtained for blackcurrant (1.39% error); the classification performance was similar for chokeberry and strawberry with the same error rate of 4.17%. The highest error of 6.94% was obtained for raspberry beverages.

Comparison of the classification models

Based on the present results, we may conclude that PLS-DA analysis of uEEMs and uTSFS provided similar overall classification performance. Both measurement techniques provided comprehensive characterization of the samples studied, and contained similar analytical information. On the other hand, EEMs may have some advantages in explorative studies. Thanks to their trilinear structure, the use of PARAFAC analysis allows the extraction of unique spectral profiles of the fluorescent components, facilitating or extending the possibilities of interpretation. TSFS techniques may have same advantages in practical applications, like elimination of Rayleigh scattering and simplified data analysis.

Interestingly, very similar overall classification results were obtained for the analysis of uTSFS and SFS ($\Delta\lambda = 40$ nm). Due to the simultaneous scanning of excitation and emission monochromators, even a single synchronous fluorescence spectrum provides information about all of the fluorescent components present in a sample. However, as apparent in our results, Figure 6, the choice of $\Delta\lambda$ affects the classification results quite markedly. Generally, the lower $\Delta\lambda$ values should provide better resolution of bands from different fluorophores due to their narrowing. However, the optimal $\Delta\lambda$ value for the particular compounds is defined by

their Stokes shift, thus different fluorophores have their maxima at different $\Delta\lambda$ values. Based on the results of this study and other published results, we conclude that an optimal value of $\Delta\lambda$ should be selected empirically for a particular system and problem studied.

The classification performance of models based on the individual EMS was lower as compared to the entire uEEMs, uTSFS and single SFS. This is due to the inherent characteristics of this type of spectrum, which contain overlapping signals originating from those particular fluorophores, which are excited at the selected wavelength. Thus, in a single EMS, part of the analytical information may be lost in a multifluorophoric system. Similarly, poorer classification results for the analysis of individual EMS as compared to the uEEMs-PCA-LDA and SFS-PCA-LDA results were obtained recently in the classification of brandy according to the region of production [40].

The lowest classification performance was presently obtained in the PARAFAC-PLS-DA model. These results may be due to the some limitations of this model. The analysis of all of the EEMs resulted in a model with four components, each of those most probably representing a group of fluorescent components with a similar spectral profile, rather than an individual chemical compound. Thus, although PARAFAC decomposition provided valuable insights into the spectral interpretation and identification of fluorophores, some of the information important for the sample differentiation and classification was lost.

4. Conclusions

The EEMs and TSFS provide the overall characteristics of the natural fluorescence of berry fruit juices. The analysis of the EEMs using PARAFAC extracted four fluorescent components and revealed some differences among the fluorescence of the beverages obtained from different fruit. The beverages originated from different fruit were successfully classified on the basis of their fluorescence using the PLS-DA method. Good PLS-DA results were

obtained for both the analysis of unfolded matrices obtained using multidimensional fluorescence techniques as well as for individual SFS and conventional EMS. The optimal parameters should be carefully selected for the discrimination purposes, namely λ_{exc} for EMS and $\Delta\lambda$ for the SFS measurements, as they significantly affected the model performance. This selection may be important for the potential practical applications, for fluorescence screening of juices for authenticity.

The presented results show usability of fluorescence for identifying the berry species used to prepare berry beverages. These results may be potentially useful for the development of rapid and reagent-free methods for authenticity testing of berry beverages.

Conflict of interest statement

The authors have declared that no conflicting interests exist.

Acknowledgements

Grant 2016/23/B/NZ9/03591 from the National Science Centre, Poland, is gratefully acknowledged.

References

1. Ellis D I, Muhamadali H, Haughey S A, Elliott C T and Goodacre R 2015 Point-and-shoot: Rapid quantitative detection methods for on-site food fraud analysis - moving out of the laboratory and into the food supply chain *Anal. Methods-UK* **7** 9401-14
2. Christensen J, Norgaard L, Bro R and Engelsen S B 2006 Multivariate autofluorescence of intact food systems *Chem. Rev.* **106** 1979-94
3. Lloyd J B F 1971 Synchronized excitation of fluorescence emission spectra *Nature-Physical Science* **231** 64-65

4. Andrade-Eiroa Á, de-Armas G, Estela J-M and Cerdà V 2010 Critical approach to synchronous spectrofluorimetry. I *TrAC, Trends Anal. Chem.* **29** 885-901

5. Kumar K, Tarai M and Mishra A K 2017 Unconventional steady-state fluorescence spectroscopy as an analytical technique for analyses of complex-multifluorophoric mixtures *TrAC, Trends Anal. Chem.* **97** 216-43

6. Sikorska E, Khmelinskii I and Sikorski M Fluorescence spectroscopy and imaging instruments for food quality evaluation, in: J. Zhong, X. Wang (Eds), Evaluation technologies for food quality, Woodhead Publishing; 2019. pp. 491-533

7. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T and Sochor J 2015 Bioactive compounds and antioxidant activity in different types of berries *Int. J. Mol. Sci.* **16** 24673

8. Hidalgo G-I and Almajano M P 2017 Red fruits: Extraction of antioxidants, phenolic content, and radical scavenging determination: A review *Antioxidants* **6** 7

9. Veberic R, Slatnar A, Bizjak J, Stampar F and Mikulic-Petkovsek M 2015 Anthocyanin composition of different wild and cultivated berry species *LWT - Food Sci. Technol.* **60** 509-17

10. Di Vittori L, Mazzoni L, Battino M and Mezzetti B 2018 Pre-harvest factors influencing the quality of berries *Sci. Hortic. Amsterdam* **233** 310-22

11. Manganaris G A, Goulas V, Vicente A R and Terry L A 2014 Berry antioxidants: Small fruits providing large benefits *J. Sci. Food. Agric.* **94** 825-33

12. Szajdek A and Borowska E J 2008 Bioactive compounds and health-promoting properties of berry fruits: A review *Plant Food. Hum. Nutr.* **63** 147-56

13. Obón J M, Díaz-García M C and Castellar M R 2011 Red fruit juice quality and authenticity control by HPLC *J. Food. Compos. Anal.* **24** 760-71

14. Zhang J, Yu Q, Cheng H, Ge Y, Liu H, Ye X and Chen Y 2018 Metabolomic approach for the authentication of berry fruit juice by liquid chromatography quadrupole time-of-flight mass spectrometry coupled to chemometrics *J. Agric. Food. Chem.* **66** 8199-208

15. Barbosa S, Pardo-Mates N, Hidalgo-Serrano M, Saurina J, Puignou L and Núñez O 2018 Detection and quantitation of frauds in the authentication of cranberry-based extracts by UHPLC-HRMS (Orbitrap) polyphenolic profiling and multivariate calibration methods *J. Agric. Food. Chem*. **66** 9353-65

16. Wu Y, Li M, Yang Y, Jiang L, Liu M, Wang B and Wang Y 2018 Authentication of small berry fruit in fruit products by DNA barcoding method *J. Food. Sci.* **83** 1494-504

17. Saad R, Bouveresse D J-R, Locquet N and Rutledge D N 2016 Using pH variations to improve the discrimination of wines by 3D front face fluorescence spectroscopy associated to independent components analysis *Talanta* **153** 278-84

18. Airado-Rodríguez D, Durán-Merás I, Galeano-Díaz T and Wold J P 2011 Front-face fluorescence spectroscopy: A new tool for control in the wine *industry J. Food Compos. Anal.* **24** 257-64

19. Airado-Rodríguez D, Galeano-Díaz T, Durán-Merás I and Wold J P 2009 Usefulness of fluorescence excitation−emission matrices in combination with PARAFAC, as fingerprints of red wines *J. Agric. Food. Chem.* **57** 1711-20.

20. Azcarate S M, de Araújo Gomes A, Alcaraz M R, Ugulino de Araújo M C, Camiña J M and Goicoechea H C 2015 Modeling excitation–emission fluorescence matrices with pattern recognition algorithms for classification of Argentine white wines according grape variety *Food Chem.* **184** 214-9

21. Sádecká J, Jakubíková M and Májek P 2018 Fluorescence spectroscopy for discrimination of botrytized wines *Food Control* **88** 75-84

22. Schueuermann C, Silcock P and Bremer P 2018 Front-face fluorescence spectroscopy in combination with parallel factor analysis for profiling of clonal and vineyard site differences in commercially produced Pinot Noir grape juices and wines *J. Food Compos. Anal.* **66** 30-8

23. Clément A, Panneton B, Bastien R and Fernandez P 2017 Ice cider fingerprinting using optical spectroscopy and simple laboratory measurements *J. Food. Eng.* **204** 55-63

24. Markechová D, Májek P and Sádecká J 2014 Fluorescence spectroscopy and multivariate methods for the determination of brandy adulteration with mixed wine spirit *Food Chem.* **159** 193-9

25. Sádecká J, Uríčková V and Jakubíková M. Fluorescence spectroscopy for the analysis of spirit drinks, in: M.T. Stauffer (Ed.), Applications of molecular spectroscopy to current research in the chemical and biological sciences, InTech, Rijeka, 2016. pp. 339-362.

26. Poryvkina L, Tsvetkova N and Sobolev I 2014 Evaluation of apple juice quality using spectral fluorescence signatures *Food Chem.* **152** 573-7

27. Seiden P, Bro R, Poll L and Munck L 1996 Exploring fluorescence spectra of apple juice and their connection to quality parameters by chemometrics *J. Agric. Food Chem.* **44** 3202-5

28. Włodarska K, Khmelinskii I and Sikorska E 2018 Authentication of apple juice categories based on multivariate analysis of the synchronous fluorescence spectra *Food Control* **86** 42-9

29. Ammari F, Redjdal L and Rutledge D N 2015 Detection of orange juice frauds using front-face fluorescence spectroscopy and independent components analysis *Food Chem.* **168** 211-7.

30. Dankowska A, Domagała A and Kowalewski W 2017 Quantification of coffea arabica and coffea canephora var. Robusta concentration in blends by means of synchronous fluorescence and UV-VIS spectroscopies *Talanta* **172** 215-20

31. Seetohul L N, Scott S M, O'Hare W T, Ali Z and Islam M 2013 Discrimination of Sri Lankan black teas using fluorescence spectroscopy and linear discriminant analysis *J. Sci. Food Agric.* **93** 2308-14

32. Dong Y, Liu X, Mei L, Feng C, Yan C and He S 2014 LED-induced fluorescence system for tea classification and quality assessment *J. Food Eng.* **137** 95-100

33. Dankowska A and Kowalewski W 2019 Tea types classification with data fusion of UV–VIS, synchronous fluorescence and NIR spectroscopies and chemometric analysis *Spectrochim. Acta A* **211** 195-202

34. Andersen C M and Bro R 2003 Practical aspects of PARAFAC modeling of fluorescence excitation-emission data *J. Chemom.* **17** 200-15

35. Ballabio D and Consonni V 2013 Classification tools in chemistry. Part 1: Linear models. PLS-DA *Anal. Methods-UK* **5** 3790-8

36. Chong I-G and Jun C-H 2005 Performance of some variable selection methods when multicollinearity is present *Chemometr. Intell. Lab.* **78** 103-12

37. Agati G, Matteini P, Oliveira J, de Freitas V and Mateus N 2013 Fluorescence approach for measuring anthocyanins and derived pigments in red wine *J. Agric. Food Chem.* **61** 10156-62

589 38. García-Plazaola J I, Fernández-Marín B, Duke S O, Hernández A, López-Arbeloa F
590 and Becerril J M 2015 Autofluorescence: Biological functions and technical
591 applications *Plant Sci.* **236** 136-45
592 39. Mezzetti A, Protti S, Lapouge C and Cornard J-P 2011 Protic equilibria as the key
593 factor of quercetin emission in solution. Relevance to biochemical and analytical
594 studies *Phys. Chem. Chem. Phys.* **13** 6858-64
595 40. Sádecká J, Uríčková V, Májek P and Jakubíková M 2019 Comparison of different
596 fluorescence techniques in brandy classification by region of production *Spectrochim.*
597 *Acta A* **216** 125-35
598
599

Table caption

Table 1. Characteristics of the classification models, and cross-validation results: average classification error, classification errors, sensitivity and specificity for the four classes in the classification models: chokeberry, blackcurrant, raspberry and strawberry beverages.

Figures captions

Figure 1. Excitation-emission matrices of juices from different berry fruit: A) chokeberry, B) blackcurrant, C) strawberry and D) raspberry.

Figure 2. Total synchronous fluorescence spectra of juices from different berry fruit: A) chokeberry, B) blackcurrant, C) strawberry and D) raspberry. The spectra for the same samples are presented as those in Figure 1.

Figure 3. Results of PARAFAC of EEMs: A) excitation profiles, B) emission profiles, C) scores on component 1 vs component 2 and D) scores on component 3 vs component 4.

Figure 4. Variables in the projection for the uEEMs-PLS-DA model (normalized) for each of the classes: A) chokeberry, B) blackcurrant, C) strawberry, and D) raspberry.

Figure 5. Variables in the projection for the uTSFS-PLS-DA model (normalized) for each of the classes: A) chokeberry, B) blackcurrant, C) strawberry, and D) raspberry.

Figure 6. Classification error for the PLS-DA classification models based on A) single (normalized) emission spectra, and B) single synchronous fluorescence spectra. The numbers represent number of latent variables for PLS-DA models.

Table 1. Characteristics of the classification models, and cross-validation results: average classification error, classification errors, sensitivity and specificity for the four classes in the classification models: chokeberry, blackcurrant, raspberry and strawberry beverages.

Parameter	Class	PARAFAC-PLS-DA[1]	uEEMs-PLS-DA[2]	uTSFS-PLS-DA[3]	EM-PLS-DA[4]	SFS-PLS-DA[5]
Number of latent variables		3	7	7	7	8
Average classification error (%)		15.27	4.86	4.86	7.64	4.42
Classification error (%)	Chokeberry	6.94	6.94	1.39	2.8	4.17
	Blackcurrant	27.77	5.55	6.94	2.8	1.39
	Raspberry	18.05	6.94	8.33	12.5	6.94
	Strawberry	8.33	0.00	2.78	12.5	4.17
Sensitivity (%)	Chokeberry	91.7	91.7	100	100	91.7
	Blackcurrant	83.3	91.7	91.7	100	100
	Raspberry	75.0	91.7	91.7	83.3	91.7
	Strawberry	91.7	100	100	91.7	91.7
Specificity (%)	Chokeberry	94.4	94.4	97.2	94.4	100
	Blackcurrant	61.1	97.2	94.4	94.4	97.2
	Raspberry	88.9	94.4	91.7	91.7	94.4
	Strawberry	91.7	100	94.4	83.3	100

[1] – PARAFAC model with 4 components, [2] – normalized uEEMs, [3] – normalized uTSFS, [4] – normalized emission spectra measured at 360 nm excitation wavelength, [5] – synchronous fluorescence spectra recorded at $\Delta\lambda$=40 nm.

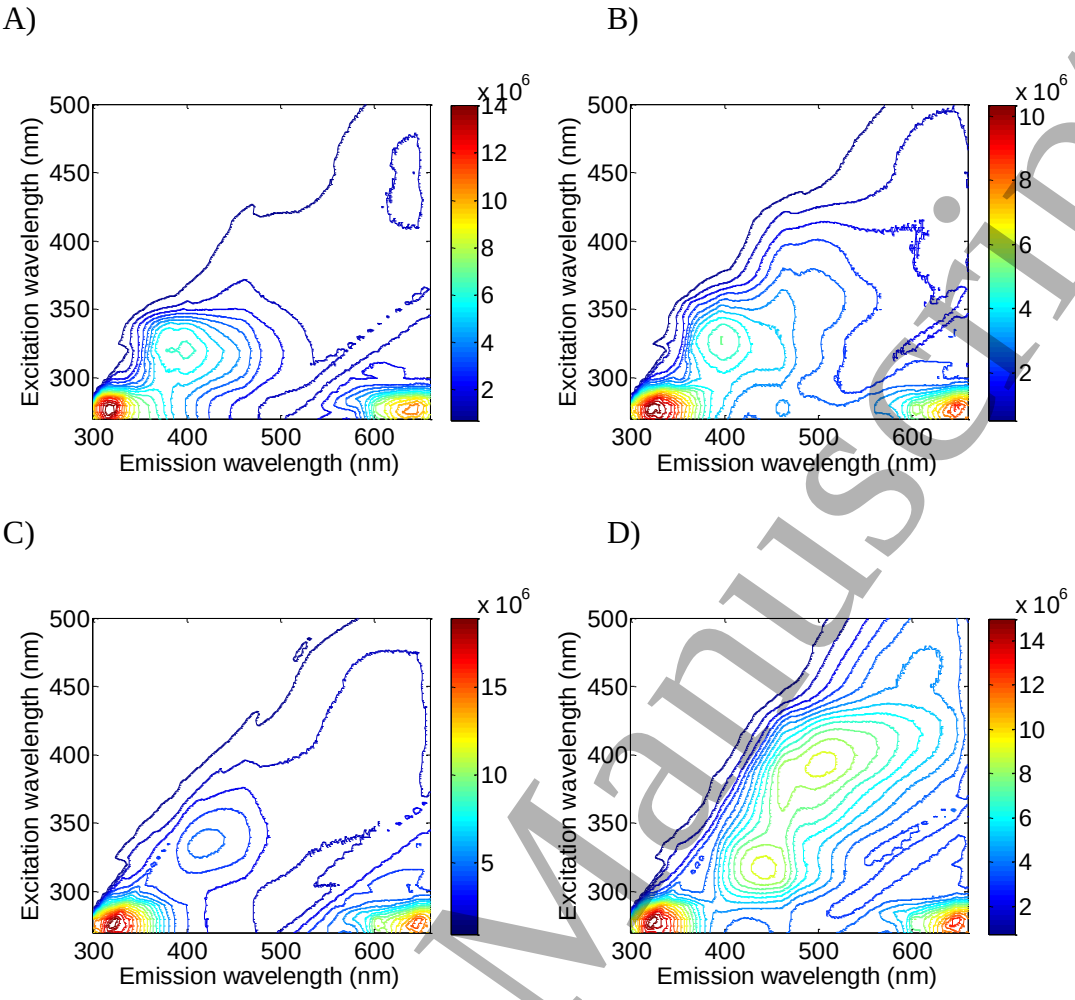


Figure 1.

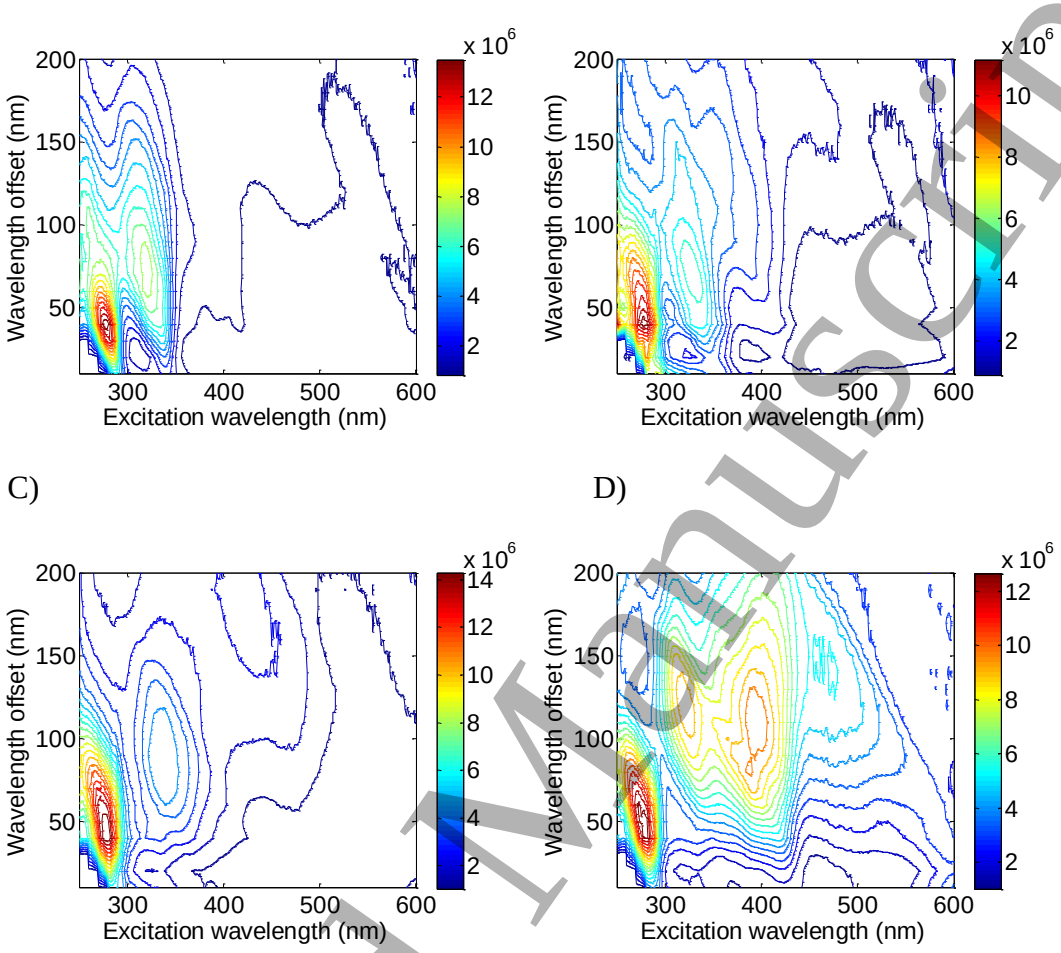


Figure 2.

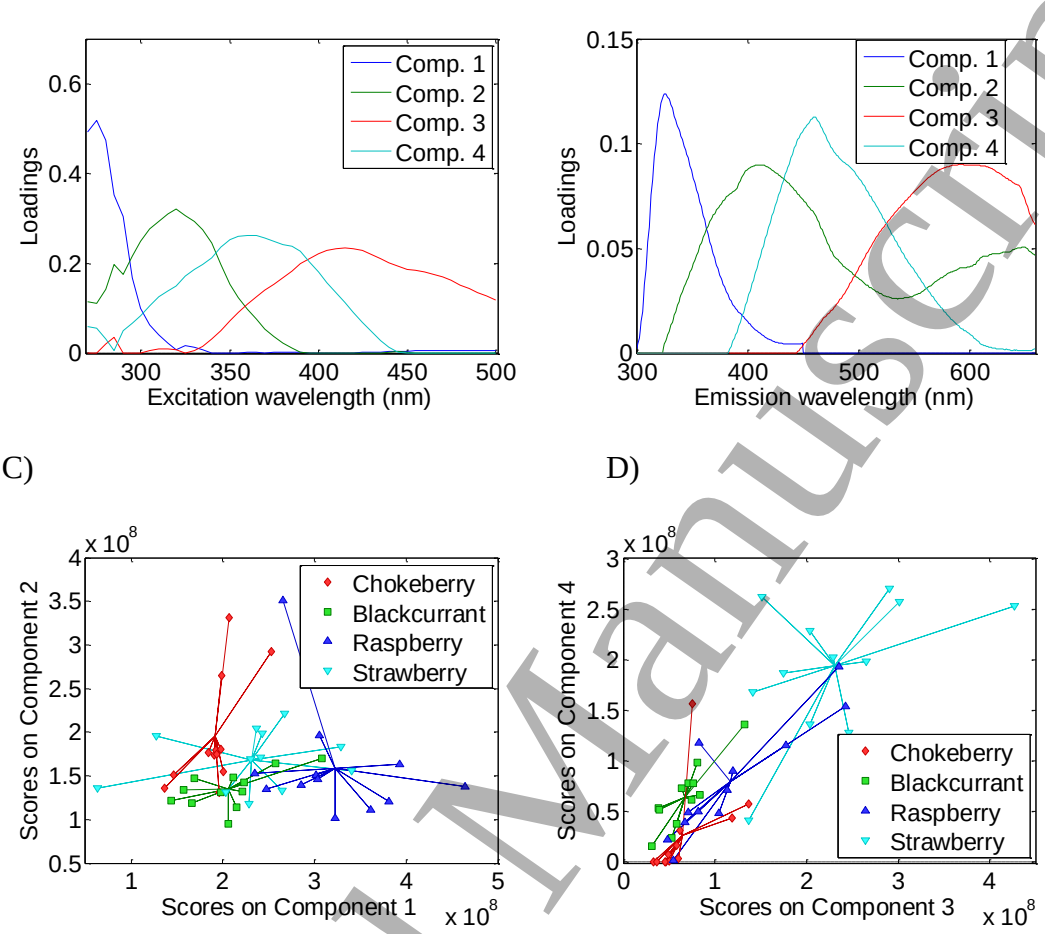


Figure 3.

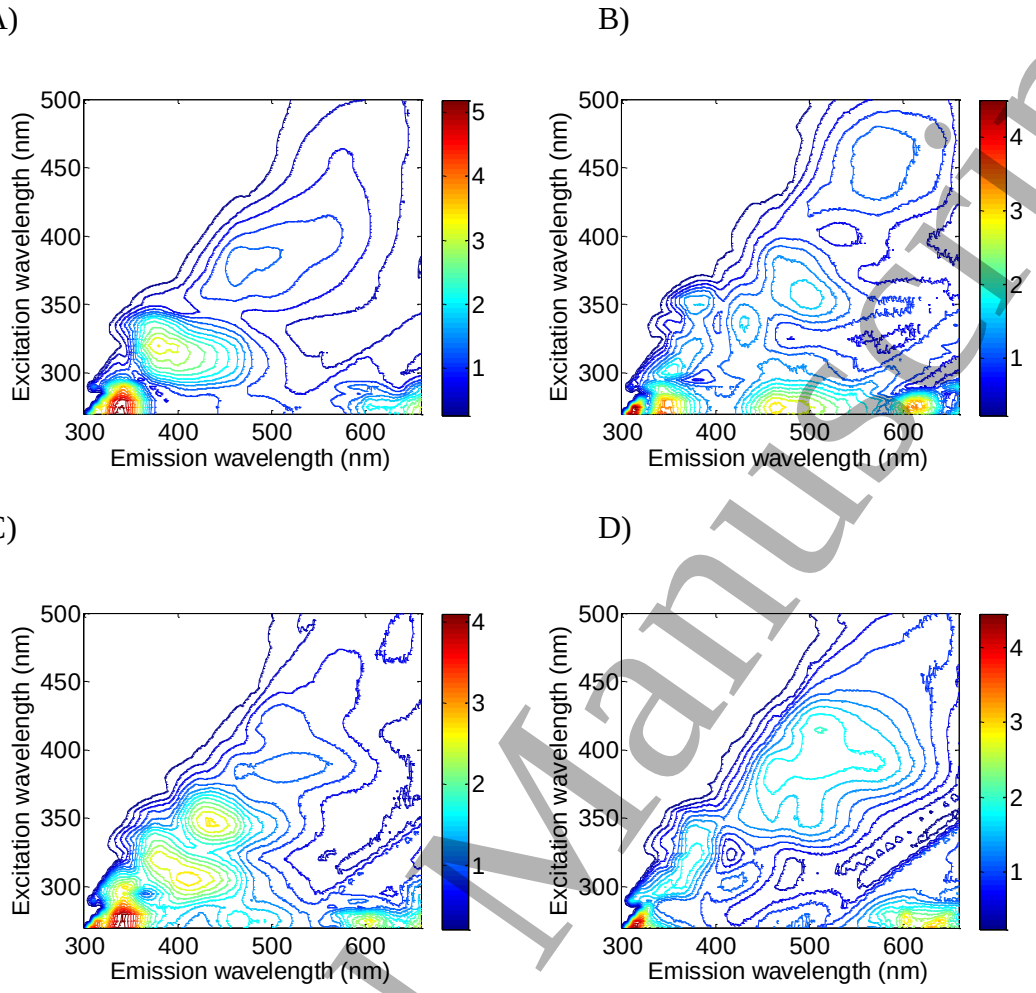


Figure 4.

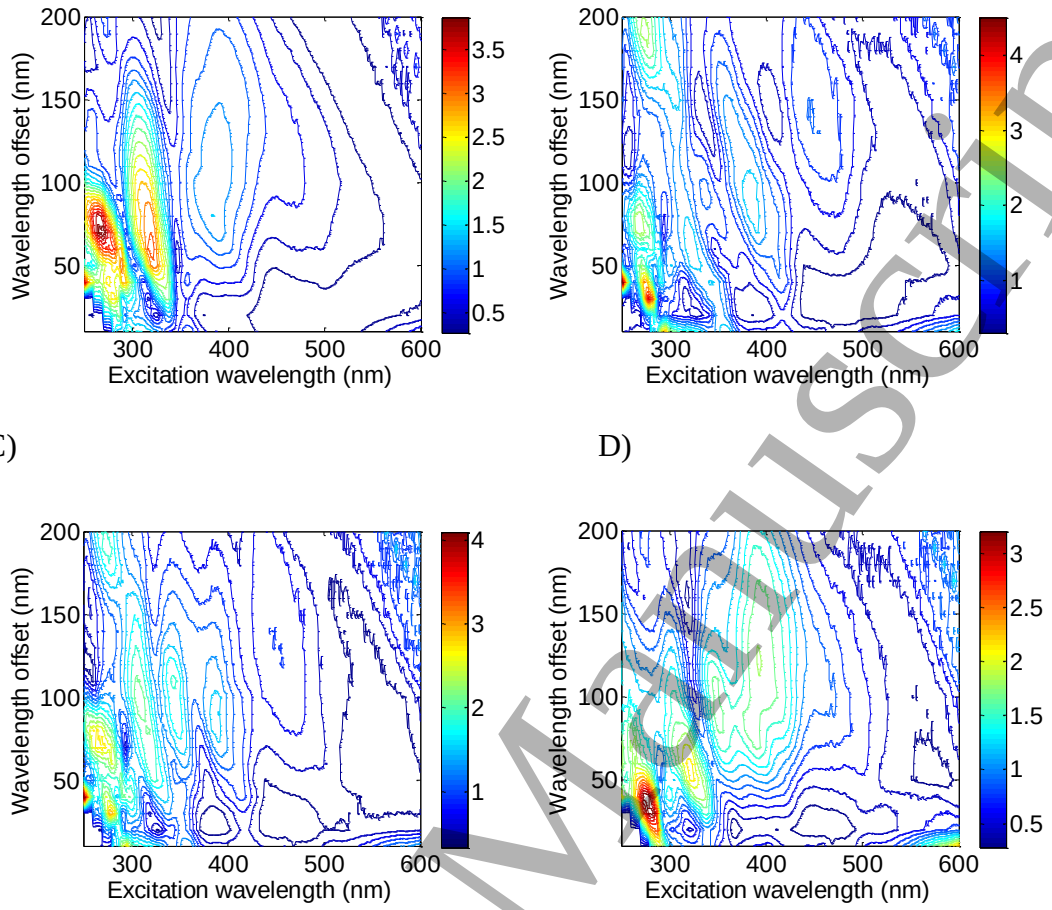
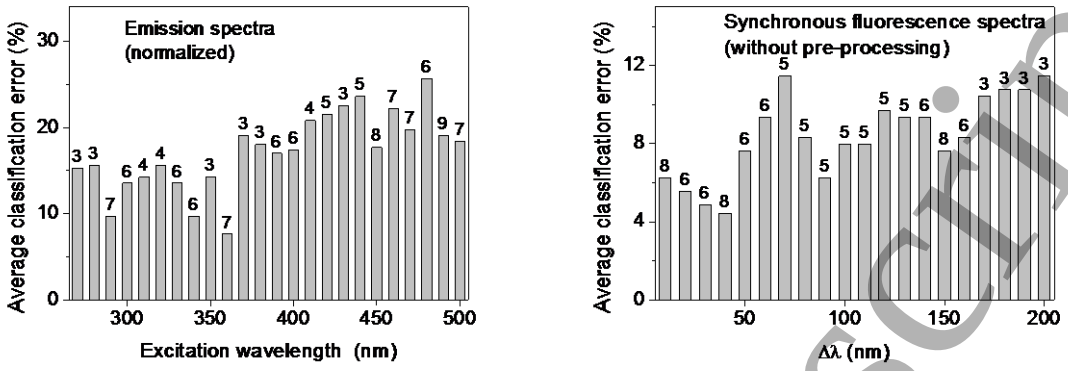


Figure 5.

675

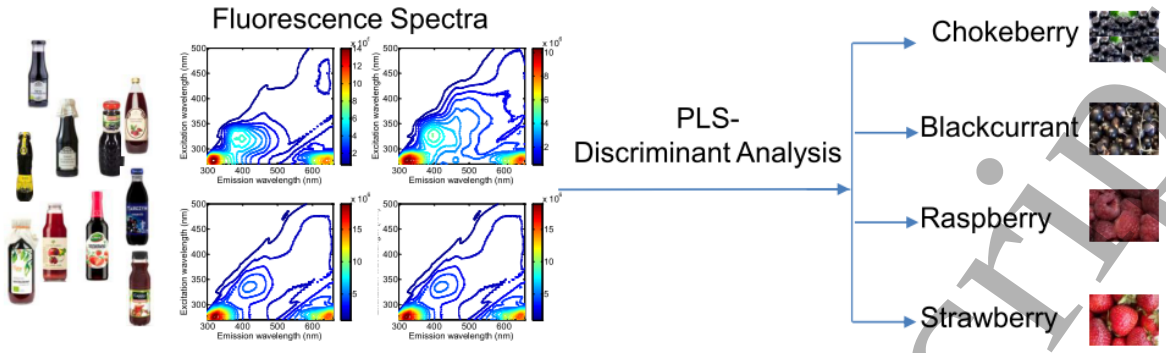
676 A) B)



677

678 **Figure 6.**

679



Graphical abstract

Highlights

- Fluorescence of chokeberry, blackcurrant, raspberry and strawberry beverages characterized
- Partial least squares – discriminant analysis (PLS-DA) applied to fluorescence data
- Beverages classified according to the fruit species with the average error below 5%
- Performance of various fluorescence techniques compared