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Explorative study of apple juice fluorescence in relation to antioxidant properties

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ABSTRACT

Total fluorescence spectra (excitation-emission matrices, EEM) were recorded for a series of commercial apple juices, including clear and cloudy juices produced from concentrate, cloudy juices that were not from concentrate, and freshly squeezed juices. An exploratory study of the spectra with parallel factor analysis (PARAFAC) revealed three groups of fluorophores with different emission properties, and these properties were characterized by excitation/emission maxima at 270/315 nm, (310, 370)/455 nm, and 430/(550, 680) nm, respectively.

A regression analysis of the total fluorescence spectra arranged into three-way arrays using *N*-way partial least squares regression methods (*N*PLS1 and *N*PLS2) and an analysis of the unfolded spectra by partial least squares methods (PLS1 and PLS2) revealed quantitative relations between the fluorescence and antioxidant properties of juices. The best models for the total phenolic contents and total antioxidant capacities were obtained by applying the *N*PLS1 method to the EEM. The model parameters were as follows: $R^2_{CV} = 0.802$, RPD = 2.3 for the total phenolic content and $R^2_{CV} = 0.808$ and RPD = 2.3 for the total antioxidant capacity. These results show the potential use of fluorescence spectroscopy for screening apple juices for their antioxidant properties.

1. Introduction

Because of their widespread consumption, apples and apple juices are among the major dietary sources of phytochemicals, including phenolics. Phenolic compounds account for the metabolic activity and antioxidant properties of plant-based foods and for putative health benefits in humans. The consumption of apples and apple products has been positively associated with a reduction in the risk of developing several diseases (Candrawinata, Golding, Roach, & Stathopoulos, 2013). Moreover, phenols affect the organoleptic properties of foods, including their colours, flavours, astringency, and hardness (Robbins, 2003).

Phenolic compounds are distributed differently throughout the apple fruit. The contents and profiles of phenolic compounds vary depending on the apple variety and environmental and post-harvest factors (the fruit season, fruit maturity, light exposure, and storage) (Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe, & Lewandowski, 2014). Fresh apples and juices differ in their phenolic contents and composition. The processing of apples during juice production significantly reduces their phenolic contents and antioxidant activities. The phenolic content of an apple juice may also change during storage as a result of both oxidative and non-oxidative degradation (Candrawinata et al., 2013).

Apples contain various phenolic compounds, including hydroxybenzoic acids (p-hydroxybenzoic, protocatechuic, gallic, syringic, and gentisic acids), hydroxycinnamic acids and their derivatives (p-coumaric, caffeic, ferulic, and chlorogenic acids), flavonols (quercetin, which is present in glycosylated forms), dihydrochalcones (phloridzin and its derivatives), anthocyanids (cyanidines and their glycosides), monomeric flavanols (epicatechin and catechin), and oligomeric flavanols (procyanidins). Normally, phenolic antioxidants determine the antioxidant activity of apples, and the contribution of vitamin C is small (Kalinowska et al., 2014).

A variety of methods have been used to determine the phenolic contents and antioxidant activities of foods (Craft, Kerrihard, Amarowicz, & Pegg, 2012; Ignat, Volf, & Popa, 2011). HPLC coupled with detection by absorbance, mass spectrometry or fluorimetry is one of the most frequently used methods to quantify individual phenolics (Corradini et al., 2011; Pyrzynska & Sentkowska, 2014). Other useful instrumental techniques involving separation include

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high-speed counter-current chromatography, supercritical fluid chromatography, gas chromatography, and capillary electrophoresis (Ignat et al., 2011).

In foods, the total phenolic content and antioxidant capacity are often quantified rather than the concentration of individual phenolic compounds. Despite their inherent limitations, these assays are often used in research (Craft et al., 2012; Ignat et al., 2011).

Several spectrophotometric methods are used to quantify the total phenolic contents and individual classes of phenolic compounds. Among these approaches, the Folin-Ciocalteu assay is used widely to determine the total phenolics (Ignat et al., 2011).

Several methods have been proposed for measuring the antioxidant activity of individual compounds and the antioxidant capacity of foods (Craft et al., 2012). These commonly used assays measure the Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), ferric-reducing ability of plasma (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical cation ($DPPH^{\cdot}$) assay (Craft et al., 2012). All these methods rely on measurements of the ability of a food or food component to scavenge specific free radicals or reduce other chemicals. The measured activity is calibrated against that of a reference compound, usually Trolox (a water-soluble derivative of vitamin E), gallic acid, or catechin.

Spectroscopic techniques coupled to chemometrics might provide an alternative to conventional methods in routine, high-throughput determinations of phenolic compounds and antioxidant capacities of foods. NIR and MIR spectroscopy coupled with chemometrics have been used to assess both the concentrations of the bioactive components and the total antioxidant capacities of foods (Lu & Rasco, 2011). For example, NIR spectroscopy and multivariate regression have been used to predict the polyphenol contents of apples (Giovanelli, Sinelli, Beghi, Guidetti, & Casiraghi, 2014; Pissard et al., 2013; Schmutzler & Huck, 2014).

Some of the phenolic compounds are fluorescent, and their contributions to the autofluorescence of food products has been reported, including that of apple juices (Poryvkina, Tsvetkova, & Sobolev, 2014; Seiden, Bro, Poll, & Munck, 1996), wine (Airado-Rodríguez, Durán-Merás, Galeano-Díaz, & Wold, 2011; Airado-Rodríguez, Galeano-Díaz, Durán-Merás, & Wold, 2009), honey (Karoui, Dufour, Bosset, & De Baerdemaeker, 2007; Lenhardt, Bro, Zeković, Dramićanin, & Dramićanin, 2015; Sergiel, Pohl, Biesaga, & Mironczyk, 2014) and strawberry fruits (Wulf et al., 2008). Recently, fluorescence spectroscopy coupled with multivariate regression was used to screen the antioxidant contents and the antioxidant capacities of coffee, peppermint extract and tomato paste (Orzel & Daszykowski, 2014; Orzel, Stanimirova, Czarnik-Matusewicz, & Daszykowski, 2015). Synchronous fluorescence spectroscopy was used to determine the phenolic acids and scopoletin in brandies (Žiak, Sádecká, Májek, & Hroboňová, 2014). These studies show that because of its high selectivity and sensitivity, fluorescence may be a valuable alternative to absorption spectroscopic techniques for quantifying the phenolic compounds in foods.

Several apple juice studies have successfully employed fluorescence. Fluorescence spectra were correlated with the soluble solid contents of apple juice (Seiden et al., 1996). Juices from two apple varieties were classified on the basis of their fluorescence (Seiden et al., 1996). This classification was also performed for apple juices that had been processed using different techniques (Poryvkina et al., 2014). Fluorescence was also correlated with newly formed products during the thermal processing of apple juices, supporting the usefulness of this parameter for monitoring the non-enzymatic browning of juice (Cohen, Birk, Mannheim, & Saguy, 1998; Zhu, Ji, Eum, & Zude, 2009).

To the best of our knowledge, there are no quantitative studies of apple juice fluorescence in relation to phenolic contents and antioxidant capacities. Thus, the aim of this study was to explore the relations between the natural fluorescence of apple juices and their total phenolic contents (TPC) and total antioxidant capacities (TAC). Spectrophotometric assays were used as reference methods to determine the TPC and TAC.

The total fluorescence spectra (excitation-emission matrices, or EEM) were recorded for each juice, and a parallel factor analysis (PARAFAC) was used in this exploratory study. Regression analyses using partial least squares (PLS) and *N*-way partial least squares (*N*PLS) were employed to investigate the relations between the fluorescence spectra of juices and TPC and TAC.

2. Materials and methods

2.1. Reagents

Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) and 20% sodium carbonate (POCh, Gliwice, Poland) were used to determine the total phenolic contents, with gallic acid (Sigma-Aldrich, Steinheim, Germany) as a reference.

For the TEAC assay, the $ABTS^{\cdot+}$ radical cation was generated using ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] diammonium salt from Roche (Mannheim, Germany) and potassium persulphate from Fluka (Buchs, Switzerland). Trolox (6-hydroxy-2,5,7,8-tertamethylchroman-2-carboxylic acid) was purchased from Sigma-Aldrich (Steinheim, Germany) and used as a standard.

2.2. Apple juices

Thirty apple juices that are available on the Polish market were evaluated in this study. These samples included juices from concentrate that were clear and cloudy, and not-from-concentrate juices that were pasteurized naturally cloudy and freshly squeezed. Juices from 18 different producers were studied; some of these samples were studied in duplicate (the samples were taken from two different production batches). The studied samples include juices that were produced from concentrate, clear (group A – 11 samples: A1a, A1b, A2, A3a, A3b, A4a, A4b, A5a, A5b, A6a, and A6b) and cloudy (group B – 2 samples: B1 and B2), and not-from-concentrate, including pasteurized naturally cloudy juices (group C – 13 samples: C1a, C1b, C2a, C2b, C3a, C3b, C4a, C4b, C5a, C5b, C6a, C6b, and C7) and freshly squeezed juices (group D – 4 samples: D1a, D1b, D2a, and D2b). Samples from the same producers are denoted with the same capital letter and number, and lower-case letters distinguish the different production batches.

2.3. Determination of the chemical parameters

2.3.1. Total phenolic content (TPC)

The TPC was determined according to Singleton and Rossi (1965), and the method was adapted to 48-well microplates. This method is based on the absorbance measurement of the reaction product at 765 nm, which is used to quantify the reduction of Folin-Ciocalteu reagent. In brief, 0.010 mL of each juice sample was diluted 1:2 with water and mixed with 0.050 mL of the Folin-Ciocalteu reagent. After 3 min, 0.15 mL of 20% sodium carbonate and 0.79 mL of deionized water were added and the solution was mixed well. After 120 min in the dark at room temperature, the absorbance was measured using an EpochTH microplate spectrophotometer (BioTek). The TPC was expressed as mg of gallic acid equivalents per litre of juice (mg GAE/L). These analyses were made at least in triplicate.

2.3.2. The total antioxidant capacity (TAC)

The TAC of juices was determined using the TEAC assay according to Re et al. (1999), as described in detail in Gliszczyńska-Świgło et al. (2006). This method is based on the absorption decay of the $ABTS^{\cdot+}$ radical cation at 734 nm, with the increase of the juice/Trolox concentration. The TEAC value was calculated from the linear regression coefficient of the calibration curve for six dilutions of the juice, as calibrated against the linear regression coefficient of the Trolox curve. The $ABTS^{\cdot+}$ cation radical was generated by tracking the interaction of 7.7 mg of ABTS that was dissolved in 1.8 mL of deionized water and 0.2 mL of 0.0069 g/mL potassium persulphate. The cation was incubated in the dark at room temperature for 16 h. The $ABTS^{\cdot+}$ cation radical working solution was diluted with methanol to an absorbance of 0.80 at 734 nm. The absorbance was recorded 6 min after mixing 0.008 mL of juice with 0.792 mL of the $ABTS^{\cdot+}$ working solution. The TEAC value was expressed in mmol of Trolox per litre of juice (mM), with Trolox being used as a standard in this assay. These measurements were performed on a Spectronic Genesys 2 spectrophotometer (Milton Roy). All determinations were performed in triplicate.

2.4. Fluorescence measurements

The fluorescence spectra were recorded using a Fluorolog 3-11 spectrofluorometer (Spex-Jobin Yvon, France). The total fluorescence spectra (excitation-emission matrices, EEM) were obtained by recording the emission spectra in the 260–700 nm range with the excitation in the 250–500 nm range, at 10 nm steps in the excitation wavelength.

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. A reference photodiode detector at the excitation monochromator stage compensated for the source intensity fluctuations. The individual spectra were corrected for the wavelength-dependent response of the system.

Front-face geometry was used for undiluted samples in a 10 mm fused-silica cuvette. Samples of clear juices were measured directly, without any treatment; samples of cloudy juices were measured after centrifugation (15,000 rpm for 5 min).

2.5. Data analysis

Pearson correlation coefficients were calculated to test for the correlations between the individual analytical parameters.

Parallel factor analysis (PARAFAC) was used to break the EEM into the contributions of the individual fluorescent components. PARAFAC is a generalization of a bilinear PCA that is expanded into three dimensions. Each PARAFAC component consists of one score vector and two loading vectors. The loading vectors correspond to the excitation and emission spectra of the individual components. The score vector contains information about the relative contribution of each component to each of the sample EEM included in the model (Andersen & Bro, 2003).

A three-way data array with a total of $30 \times 26 \times 431$ components (number of samples $\times$ number of excitation wavelengths $\times$ number of emission wavelengths) was used in the PARAFAC analysis. The Rayleigh scattering contributions to the EEM were removed by inserting the missing values into the bands centred on the wavelength identity line and the $2\lambda_{ex} = \lambda_{em}$ line. Non-negativity constraints were applied to the excitation and emission spectra and the concentrations. The core consistency diagnostic (CORCONDIA) was used for finding the optimal number of components in the PARAFAC models (Andersen & Bro, 2003).

2.5.1. Regression analysis

Partial least squares regression (PLS) and *N*-way partial least squares regression (*N*PLS) were used to establish the calibration models between the fluorescence data (the *X* matrix) and the chemical parameters (the *Y* matrix).

The PLS method models both the *X*- and *Y*-matrices simultaneously, finding the latent variables in *X* that best predict the latent variables in *Y* (Wold, Sjöström, & Eriksson, 2001). The *N*-way PLS is an extension of the two-way PLS regression to the multi-way data, where the independent parameter set is an array of more than two dimensions.

PLS was used to model the relation between the total fluorescence spectra (EEM), which is held in an unfolded array, and the chemical data. The *X* data included the entire EEM, which was arranged into a $30 \times 11{,}206$ matrix (number of samples $\times$ the number of emission wavelengths *times* the number of excitation wavelengths).

N-way PLS was used to model the relation between the total fluorescence (EEM) and the chemical data. The *X* data consisting of the entire EEM were arranged into three-way arrays with a total of $30 \times 26 \times 431$ elements (number of samples $\times$ number of excitation wavelengths $\times$ number of emission wavelengths). The Rayleigh scattering contributions to the EEM were removed by inserting the missing values into the bands centred on the wavelength identity line and the $2\lambda_{ex} = \lambda_{em}$ line.

The data pre-treatment included mean-centring over the first mode (over the sample set). Similar to the PLS1 regression, the *Y* data for the *N*-way PLS1 analysis contained the values of an individual chemical parameter (TPC or TAC). Additionally, PLS2 and *N*PLS2 analyses were performed, modelling the two analytical parameters simultaneously.

Full leave-one-out cross-validation was applied to all of the regression models. The regression models were evaluated using the determination coefficient (R^2) and the root mean-square error of the cross-validation (RMSECV) as the term for indicating the prediction error of the model. The optimal number of components was chosen as the minimum for the plot of the RMSECV as a function of the number of components. The predictive ability of the models was evaluated by finding the ratio of the standard deviation for the reference data to the RMSECV, which was designated RPD. The RPD provides a means for standardizing the RMSECV and evaluating the robustness of the model (Nicolaï et al., 2007).

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

3. Results and discussion

3.1. Total phenolic contents and total antioxidant capacities of the apple juices

The thirty apple juice samples studied here included clear (group A) and cloudy (group B) juices produced from concentrate, pasteurized naturally cloudy (group C) juices that were not produced from concentrate, and freshly squeezed juices (group D). The mean values, ranges and standard deviations of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the studied samples are presented in Table 1.

The antioxidant content and the antioxidant capacity that were determined using standard spectrophotometric methods showed significant variations within the sample set. The juices exhibit significant between-group variability in the studied parameters when the mean values were considered. However, the phenolic compound content and capacity varied significantly even within each of the groups. The observed variability likely resulted from both the production methods and the raw material composition.

Table 1
The total phenolic content (TPC) and the total antioxidant capacity (TAC) of the apple juices under study (n = 30 samples).

Juices	Number of samples	TPC (mg GAE/L)			TAC (mM)		
		Range	Mean	SD	Range	Mean	SD
All	30	174–1925	552	403	1.1–16.7	4.6	3.8
Clear from concentrate (A)	11	193–602	385	135	1.1–5.0	2.4	1.1
Cloudy from concentrate (B)	2	386, 491	439	–	4.4, 5.5	5.0	–
Cloudy not from concentrate (C)	13	174–1925	746	542	1.4–16.7	6.7	4.8
Freshly squeezed (D)	4	275–720	436	195	2.2–8.2	4.0	2.8

The lowest mean values of the TPC and TAC were obtained for the clear juices from concentrate (group A) in comparison with the cloudy juices (groups B, C, and D).

The highest values for the studied parameters were recorded for cloudy juices that were not made from concentrate (group C), with some results even exceeding those reported in the literature (Kahle, Kraus, & Richling, 2005). Several producers declared that vitamin C had been added to some juices. Thus, the added vitamin C may have contributed to the high values observed for TAC and may have affected the TPC values. It should be noted that the TPC assay, which uses the Folin-Ciocalteu reagent, is subject to interference, particularly from readily reducible compounds. Ascorbic acid (vitamin C) is therefore a confounding factor in the analysis of wine and most fruit (Craft et al., 2012).

Published data confirm the substantial variability in the quantity of phenolic antioxidants in apple juices as determined by HPLC. For example, the total phenolic contents found in laboratory-produced juices were 1044.4 mg/L and 698.5 mg/L in Champion cloudy and clear juices, and 472.2 mg/L and 250.1 mg/L in Idared cloudy and clear juices, respectively (Oszmiański, Wolniak, Wojdyło, & Wawer, 2007). For comparison, the total polyphenol contents in commercial clear juices ranged from 63.84 to 163.35 mg/L (Gliszczyńska-Świgło & Tyrakowska, 2003). In another study of commercial apple juices, the total polyphenol contents of cloudy juices (21 samples) varied between 152 and 459 mg/L, whereas in clear apple juices (3 samples), the contents ranged from 110 to 173 mg/L (Kahle et al., 2005).

A positive correlation was obtained between the TPC and TAC, with an estimated Pearson correlation coefficient of 0.944. The observed relation between TPC and TAC for apple juices is in accordance with the literature data, indicating that the antioxidant properties of the apple juices are primarily determined by their phenolic contents (Gardner, White, McPhail, & Duthie, 2000; Gliszczyńska-Świgło & Tyrakowska, 2003).

3.2. Fluorescence of apple juices

3.2.1. Total fluorescence spectra

Fig. 1 shows the EEM of four apple juices that represent each of the studied categories. Note that similar characteristic emission patterns are observed in all of the presented spectra. Specifically, four emission bands are present, and they have excitation/emission maxima in the following ranges: 260–279/313–342 nm, 308–320/438–451 nm, 364–389/451–463 nm, and 376–440/486–548 nm. The individual juices differ in terms of the exact positions of the maxima and the intensities of the particular bands. Moreover, a fifth long-wavelength band is observed with the 415–418/678–681 nm excitation/emission maxima in some of the cloudy juices only.

3.2.2. PARAFAC of the total fluorescence spectra

A more detailed insight into the fluorescence characteristics of the entire sample set was provided by a PARAFAC analysis of the EEM data. The objective of this analysis was to resolve the fluorescence landscapes into the contributions of the individual fluorescent components. Based on the core consistency and visual inspection of both the residuals and the loadings, an optimal PARAFAC model was identified as having three components (92.5% of the variance was explained, with a 79.0 core consistency value). Fig. 2 shows the excitation and emission loadings of the three extracted components and their respective score plots.

The first component had excitation/emission maxima at 270/315 nm. The second PARAFAC component was characterized by excitation spectrum with two bands displaying maxima at 310 and 370 nm and an emission band with a maximum at 455 nm. The third PARAFAC component exhibited a broad excitation spectrum with its maximum at 430 nm and emission with its maximum at 550 nm. An additional narrow band with a maximum at 680 nm was observed at the long-wavelength slope of the first band. Based on these results, it appears that the extracted PARAFAC components represented groups of chemical compounds with similar fluorescence properties rather than individual compounds.

The contributions of each of the three PARAFAC components to the spectra of the individual juices are shown in the score plots (Fig. 2c and d). The PARAFAC score values were plotted against one another (component 1 in comparison with 2, and component 2 in comparison with 3) to compare the fluorescence patterns of the studied juices. The clear juices from concentrate (group A) were characterized by the low-to-intermediate contribution of components 1, 2 and 3 relative to that of the other samples. The cloudy juices from concentrate (group B) and not-from-concentrate (group C) presented an intermediate contribution of component 1 and intermediate-to-high contributions of components 2 and 3. The fresh juices (group D) made an intermediate-to-high contribution of component 1 and relatively low contributions of components 2 and 3.

The PARAFAC score values allowed only limited discrimination among the juices according to their production method. Namely, the fresh juices (group D) were discriminated from the other three groups of juices (A, B, and C) in the plane that was defined by the first and second components. It should be noted that the juice properties are determined not only by processing but also by the raw materials; as we have stressed, similarly processed juices may present different antioxidant properties.

3.2.3. Fluorescent components in the apple juices

The assignment of the specific fluorescence bands is only tentative, and it is based on published data. Many of the native food components exhibit fluorescence (Christensen, Nørgaard, Bro, & Engelsen, 2006). Apples contain several groups of compounds that are fluorescent, including aromatic amino acids, phenolics, and vitamins B_2 and B_6 (Feliciano et al., 2010; Wu et al., 2007). Chlorogenic acid is the leading phenolic compound found in apple juices. Other phenolics that have been reported in apple juices include caffeic acid, p-coumaric acid, ferulic acid, catechin, epicatechin, procyanidins (B1, B2, C1 trimer), rutin, phloridzin, quercetin, isoquercitrin, and hyperin (Gliszczyńska-Świgło & Tyrakowska, 2003; Kahle et al., 2005; Karaman, Tütem, Sözgen Başkan, & Apak, 2010).

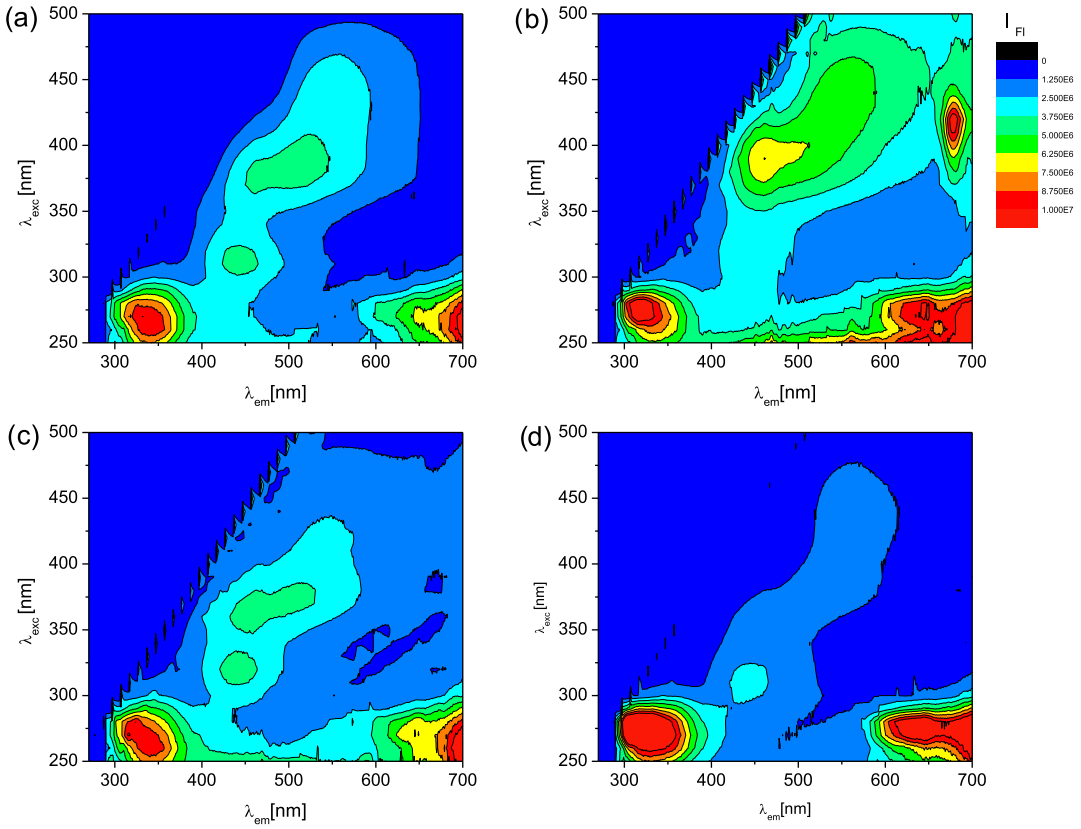

Fig. 1. The total fluorescence spectra (EEM) of the apple juices were as follows: (a) clear from concentrate (sample A1a), (b) cloudy from concentrate (sample B2), (c) cloudy not from concentrate (sample C5a), and (d) freshly squeezed (sample D2a).

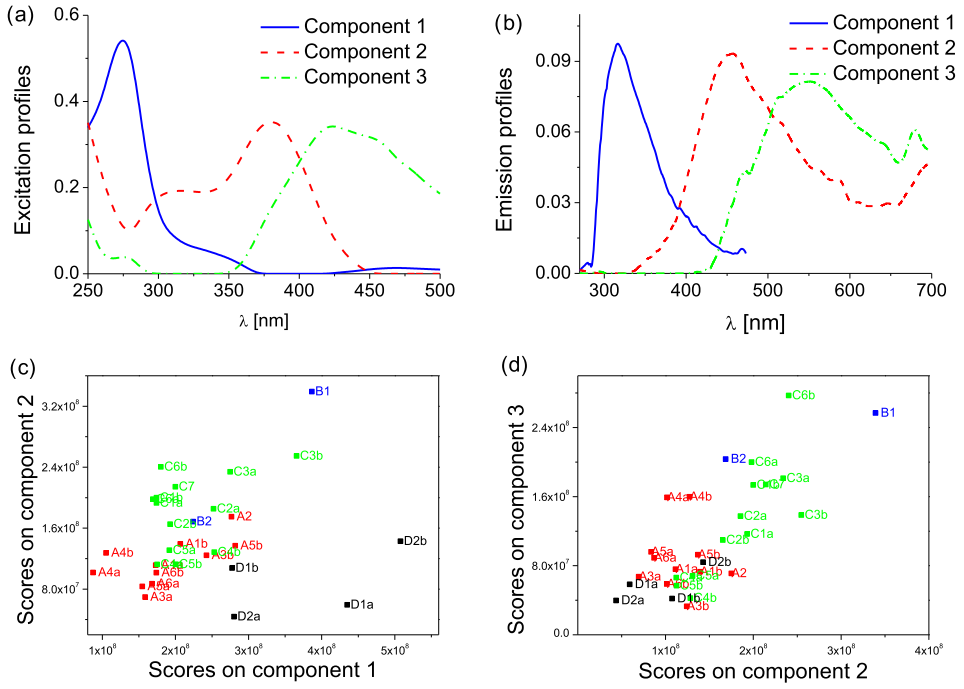

Fig. 2. Results of the PARAFAC analysis on the total fluorescence spectra (EEM) in the apple juice samples as follows: (a) excitation spectra, (b) emission spectra, (c) scores, component 1 in comparison with component 2 and (d) scores, component 2 in comparison with component 3.

The first PARAFAC component had its excitation/emission maxima at 270/315 nm. It should be noted that aqueous tyrosine solutions have excitation/emission maxima at 276/302 nm, and those of phenylalanine are 258/284 nm (Christensen et al., 2006). The presence of tyrosine and phenylalanine was reported in apples (Wu et al., 2007). Flavanols, including catechin and epicatechin,

emit at 317 and 316 nm when excited at 279 and 280 nm, respectively (Airado-Rodríguez et al., 2009).

The second PARAFAC component had an excitation spectrum with two bands centred at 310 and 370 nm and an emission band at 455 nm. According to the published data (Airado-Rodríguez et al., 2009; Airado-Rodríguez et al., 2011), the emission maxima of many different phenolic compounds are all observed in approximately the same spectral range. The excitation/emission maxima of chlorogenic acid were observed at 245, 310/445 nm (Mazina, Vaher, Kuhtinskaja, Poryvkina, & Kaljurand, 2015). When excited at 260 nm, the emission maxima of the caffeic, ferulic and p-coumaric acids were reported at 426, 400 and 422 nm, respectively (Airado-Rodríguez et al., 2011). The emission maximum for quercetin was reported at 400-420 nm, with excitation at 260-262 nm (Airado-Rodríguez et al., 2011) or at 480 nm with excitation at 427 nm in tartrate buffer (pH = 7) and 13% ethanol (Airado-Rodríguez et al., 2009). Another study found that quercetin fluorescence is pH-dependent, with dual emissions observed in aqueous solutions at pH = 5; weak fluorescence with maxima at 455 nm and 521 nm was attributed to the normal form and the tautomer formed in the excited-state proton transfer, respectively (Mezzetti, Protti, Lapouge, & Cornard, 2011).

The excitation spectrum of the second PARAFAC component may also correspond to the absorption spectra of the phenolic compounds. Phenolic acids have absorption maxima in the 200–290 nm range, and because of an additional conjugation, hydroxycinnamic acids and their derivatives have an additional broad absorbance band, extending from 270 to 360 nm (Robbins, 2003). In addition, the typical UV–vis spectra of the flavonoids include two absorbance bands in the 350–385 nm and 250–290 nm ranges (Tsimogiannis, Samiotaki, Panayotou, & Oreopoulou, 2007).

The third PARAFAC component exhibited a broad excitation spectrum with a maximum at 430 nm and an emission with a maximum at 550 nm; when there was a second narrow band on the long-wavelength slope of this band, its maximum was 680 nm (Fig. 2a and b).

The fluorescence that occurred when the excitation/emission values were 400/493 nm (Cohen et al., 1998) and 400/476 nm (Zhu et al., 2009) was associated with the products of the non-enzymatic browning of the apple juices. The aqueous solution of riboflavin exhibits fluorescence at 448/518 nm in excitation/emission (Christensen et al., 2006). Moreover, quercetin tautomer fluorescence may also contribute to this component, as indicated earlier (Mezzetti et al., 2011). The additional narrow emission band at the long-wavelength slope of the third PARAFAC component corresponds to chlorophyll, with its maximum at 680 nm.

The emissions spectra of the pure compounds recorded in pure solvents may differ from those recorded in a food product because fluorescence is highly sensitive to the local environment and is affected by the matrix properties. This situation is complicated still further by the fact that the contributions of the individual components to the overall fluorescence are determined by both their concentrations and their fluorescence quantum yields (Christensen et al., 2006). Therefore, only a tentative attribution of the specific emission bands was possible, based on the data presented here.

3.3. Multivariate calibration

Multivariate regression was used to model the relations between the fluorescence and the antioxidant properties of the juices (TPC and TAC) in a quantitative fashion. All the samples (n = 30) were used in the analysis. The statistics for the calibration data set is shown in Table 1. The regression was performed using *N*PLS for the analysis of the EEM that was arranged into a three-way array. PLS was used to analyse the unfolded EEM. Two different approaches were used, and they were based on PLS1 and *N*PLS1 that modelled each of the analytical parameters individually, and on PLS2 and *N*PLS2 that analysed all of the parameters simultaneously. The pre-processing methods (smoothing, first derivative) provided no pronounced improvement in the regression results; therefore, the raw spectra without any preprocessing were used in all the analyses. Table 2 presents the results of the multivariate calibrations.

Table 2
Characteristics of the regression models for the prediction of the total phenolic contents (TPC) and the total antioxidant activities (TAC) of the apple juices (n = 30 samples).

PLS model	Calibration			Cross-validation		
	LV	R^2	RMSEC	R^2	RMSECV	RPD
TPC						
EEM *N*PLS 1	10	0.959	78.9	0.802	179	2.3
EEM *N*PLS 2	9	0.931	104	0.639	245	1.6
Unfolded EEM PLS1	8	0.950	88.3	0.737	203	2.0
Unfolded EEM PLS2	7	0.907	120	0.602	256	1.6
TAC						
EEM *N*PLS 1	6	0.923	1.07	0.808	1.68	2.3
EEM *N*PLS 2	9	0.937	0.95	0.753	1.91	2.0
Unfolded EEM PLS1	8	0.956	0.76	0.749	1.90	2.0
Unfolded EEM PLS2	7	0.935	0.96	0.729	1.98	1.9

LV – number of latent variables used for PLS, R^2 – determination coefficient, RMSEC, RMSECV – root mean square errors of calibration and cross validation in original units: mg GAE/L for TPC, mM for TAC, RPD – residual predictive deviation.

The models were evaluated on the basis of the RMSECV and the determination coefficient, R^2. The usability of the models for predicting new samples was evaluated on the basis of the RPD value that provides a way to standardize the predictive accuracy (Nicolaï et al., 2007).

All of the calibration models confirm the correlation between the fluorescence and the parameters under study. The models that were obtained to predict the TPC and TAC usually had very similar prediction abilities. The performance of the PLS1 and *N*PLS1 models was generally better than that of their respective PLS2 and *N*PLS2 models. The performance of the *N*PLS1 and *N*PLS2 models was also better than that of the PLS1 and PLS2 models.

The optimal models for the TPC and TAC were based on the EEM, which was arranged into a three-way array and analysed using *N*PLS1. Both of these models had RPD = 2.3, corresponding to an approximate quantitative prediction accuracy (Nicolaï et al., 2007). Thus, these models may at least be used in the juice screening for the appropriate TPC and TAC values.

The regression models for TPC and TAC included a relatively high number of latent variables, likely for several reasons. Firstly, the TPC that was estimated by the Folin-Ciocalteu assay quantifies a wide group of phenolic compounds, likely with diverse fluorescent properties. In accounting for the limited specificity of this method, non-phenolic and non-fluorescent compounds may also contribute to the result (e.g., the already mentioned vitamin C). Therefore, modelling TPC on the basis of fluorescence is quite complex and involves more latent variables. Similarly, complex calibration models may also be expected for the TAC values, which are estimated from the fluorescence spectra indirectly, by using their correlation with the TPC values.

4. Conclusions

In summary, the natural fluorescence of the apple juice was correlated with its antioxidant properties. The front-face EEM spectra provided overall emissions characteristics of the juice samples. PARAFAC analysis uniquely separated the three groups of fluorescent components, which contributed to the emissions of the individual juices differently. Some characteristic emission patterns

surfaced in groups of similarly processed juices; however, the juice properties are strongly affected by those of the raw material, apart from the method used for their preparation. The PLS and *N*PLS regressions revealed the quantitative relation of the phenolic antioxidant content and antioxidant capacity with the fluorescence spectra.

The results and discussion presented here indicate directions for further studies, which should address several issues, including the unambiguous identification of the origin of the fluorescence bands, correlation of the fluorescence with the content of individual phenolic compounds, and need for additional tests on a larger number of samples to validate the prediction models.

Finally, we conclude that front-face fluorescence in association with chemometric analysis is a promising technique for the rapid screening of apple juices for antioxidant contents and antioxidant capacities.

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