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Authentication of apple juice categories based on multivariate analysis of the synchronous fluorescence spectra

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ABSTRACT

The usage of synchronous fluorescence as a means for discrimination between the commercial apple juice categories was studied. We recorded total synchronous fluorescence spectra (TSFS) of commercial apple juices belonging to the two categories: those produced directly - not from concentrate (NFC), and those reconstituted from concentrate (FC).

An exploratory study of the spectra using the principal component analysis (PCA) revealed differentiation between the juices of the two categories based on their fluorescence.

Partial least squares discriminant analysis (PLS-DA) was used for the development of the classification models. The analysis was performed for the unfolded TSFS (uTSFS) and individual synchronous fluorescence spectra (SFS) measured at particular emission-excitation wavelength offsets ($\Delta\lambda$), from 10 to 160 nm with 10 nm step. The best discrimination results were obtained for the model using uTSFS in the range of $\Delta\lambda = 30–160$ nm, with cross-validation and external validation error rates of 0.05 and 0.06, respectively. Models based on selected individual SFS also showed similarly good predictive ability, with cross-validation and external validation error rates of 0.08 and 0.05, respectively, for the SFS measured at $\Delta\lambda = 70$ and 90 nm. The analysis of significant variables using selectivity ratio (SR) suggests that the fluorescence of non-enzymatic browning products may significantly contribute to the differentiation of FC and NFC juices.

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1. Introduction

Fruit juices are widely consumed and may provide an alternative to fresh fruit in a healthy diet. Apple juice is one of the most popular and widely consumed fruit juices. It is appreciated for its pleasant taste and high nutritional value, providing a source of phenolic compounds, vitamins, minerals, and dietary fibres (Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe, & Lewandowski, 2014).

Due to high popularity and large demand, apple juices become a target for fraudulent practices (Moore, Spink, & Lipp, 2012). The fraudulent practices in the juice sector include mislabelling of product species and their geographical origin, diluting with water, addition of sugars and/or acids, replacement of high-cost juices with cheaper substitutes, originating from other fruit (Chang et al., 2016; Ogrinc, Kosir, Spangenberg, & Kidric, 2003; Wrolstad & Durst, 2006).

One example of such illegal practices is the replacement of a highly valued product by a product of a cheaper category. EU legislation defines two categories of fruit juice: “fruit juice” and “fruit juice reconstituted from concentrate” (Directive 2012/12/EU). “Fruit juice” is obtained directly from fruit. This type of juice is commonly described as “direct” or “not from concentrate” (NFC), although these names are not defined by law. “Fruit juice reconstituted from concentrate” (FC) is produced from concentrated juice by addition of water. The direct juices usually preserve more of the health-promoting properties of fresh fruit (Markowski, Baron, Le Quéré, & Płocharski, 2015) and have more attractive sensory properties as assessed by the consumers (Lee, Lusk, Mirosa, & Oey, 2016; Włodarska, Pawlak-Lemańska, Górecki, & Sikorska, 2016a), and for these reasons are generally valued higher by the consumers. Thus, juice fraud may involve replacement of an NFC juice by an FC juice.

The processing technology of apple juice strongly affects the quality of the final product (van der Sluis, Dekker, Skrede, & Jongen,

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2002). Commercial FC and NFC apple juices differ considerably in their bioactive components, including polyphenolic compounds (Kahle, Kraus, & Richling, 2005; Włodarska et al., 2016b). Direct apple juices available on the market include pasteurized and unpasteurized freshly squeezed juices. The NFC juices, obtained directly from fruit, are recognized as a premium market segment, and thus become a potential target of fraudulent practices.

Clear juices reconstituted from concentrate are the most common apple juices on the market. During their production, the components responsible for the presumable beneficial health effects are degraded, including the native pectin and phenolic compounds (van der Sluis et al., 2002). To restore the health-promoting properties, fruit puree is sometimes added to the juice produced from concentrate. Thus the FC apple juices available on the market include clear juices and those enriched with pulp.

Obviously fraudulent practices affect the quality and safety of the original products. The control of food authenticity is thus a necessary function of the regulatory agencies, being of great importance for quality assurance and for economic reasons (Zielinski et al., 2014). However, due to the natural variability of food and wide diversity of fraudulent practices, the development of methods for food fraud detection, including that of fruit juices, is a challenging task for applied research (Wrolstad & Durst, 2006).

Conventionally, the product authenticity is confirmed by comparison of selected physical and chemical characteristics with known reference values. Another approach is detection and quantification of the individual components, which may serve as biomarkers of an authentic product or reveal the presence of an adulterant. In particular, dihydrochalcones were proposed as the quality marker for the apple juice authentication (Versari, Biesenbruch, Barbanti, & Farnell, 1997). The chemical profiles of foods are also determined and compared, using multivariate methods with the profiles of the reference samples, enabling authentication (Zielinski et al., 2014). For example, apple juices produced from fruit from different cultivars were successfully discriminated using their chemical profiles (Campo, Santos, Berregi, & Munduate, 2005). Sugar composition (Pilando & Wrolstad, 1992) or polyphenol profiles (Guo, Yue, Yuan, & Wang, 2013) enabled discrimination of juices according to their variety and geographical origin. Commercial apple juices from different product categories were differentiated based on their chemical profiles (Włodarska et al., 2016b).

A variety of analytical techniques is used in authentication studies, including liquid and gas chromatography, nuclear magnetic resonance (NMR), mass spectrometry, stable isotope analysis, and various hyphenated techniques (Moore et al., 2012). However, these methods are time-consuming, relatively expensive, and do not always detect a specific adulteration.

Thus, non-targeted fingerprinting analysis is a valuable alternative to the chemical profiling methods. It uses fast techniques to obtain food fingerprints, which are rapidly evaluated using multivariate methods (Ellis et al., 2012). Specifically, spectroscopic techniques provide fingerprints of food samples, determined by their chemical composition and physical properties, and have been proved reliable methods in food studies including juices. Moreover, these techniques are rapid, non-destructive and inexpensive, enabling high-throughput screening for adulterations (Ellis, Muhamadali, Haughey, Elliott, & Goodacre, 2015). NMR spectroscopy is the method widely used in juice quality assessment. This technique allows both targeted and non-targeted analysis. It was successfully used for quantification of apple juice components and discrimination between FC and NFC juices (Monakhova et al., 2014).

Optical spectroscopic techniques most commonly used in food evaluation include near infrared (NIR) spectroscopy (Nicolaï et al., 2007). NIR and mid-infrared (MIR) spectroscopies were successfully used to differentiate between the apple juices from different fruit varieties (Reid, Woodcock, O'Donnell, Kelly, & Downey, 2005). NIR transflectance spectroscopy was used to detect adulteration of apple juice samples with high-fructose corn syrup and a sugar mixture (León, Kelly, & Downey, 2005). NIR spectroscopy with advanced chemometric analysis was used for detecting adulteration of apple juices (Li et al., 2017).

UV spectroscopy was used for discrimination of the apple juices from different geographical origins, for detection of apple juice adulteration with pear and sugarcane juice, and glucose, fructose, sucrose and water (Chang et al., 2016).

Fluorescence spectroscopy is gaining increasing attention in food authenticity studies (Karoui & Blecker, 2010). The main advantages of fluorescence are its higher sensitivity and selectivity, as compared to the spectroscopic absorption techniques. Fluorescence was successfully used for evaluation of various aspects of quality of the apple juices (Włodarska, Pawlak-Lemańska, Khmelinskii, & Sikorska, 2017b, 2016b; Cohen, Birk, Mannheim, & Saguy, 1998; Zhu, Ji, Eum, & Zude, 2009; Zhu, Ji, Qing, Wang, & Zude, 2011). In authentication studies, fluorescence was used for classification of juices from two different apple varieties (Seiden, Bro, Poll, & Munck, 1996). Recently, fluorescence was used for discrimination between home-pressed and commercial apple juices (Poryvkina, Tsvetkova, & Sobolev, 2014).

Synchronous scanning fluorescence technique has several advantages in providing fluorescent fingerprints of food, as compared to conventional emission spectra or excitation-emission matrices (Andrade-Eiroa, de-Armas, Estela, & Cerdà, 2010a; 2010b). This technique provides an improvement in selectivity for complex food samples, as compared to conventional measurements. It records signals of different fluorophores in a single scan, reducing overlapping of the spectral profiles, and suppressing light-scattering interferences.

Presently we explored the usage of synchronous fluorescence for the discrimination between commercial apple juices prepared directly and those reconstituted from concentrate.

2. Materials and methods

2.1. Apple juices

We studied fifty four apple juices produced in Poland and available on the Polish market. These samples included juices reconstituted from concentrate and direct juices, originating from 18 different producers. The samples were taken from three different production batches. The set of samples studied included clear (21 samples) and pulp-enriched cloudy varieties (6 samples) of juices produced from concentrate (FC) and direct, not from concentrate (NFC) juices, the latter including pasteurized naturally cloudy juices (21 samples), and freshly squeezed juices (6 samples). The authenticity of the juices studied was declared by the respective producers and taken at face value in this study.

2.2. Fluorescence measurements

The total synchronous fluorescence spectra (TSFS) were recorded using a Fluorolog 3–11 spectrofluorometer (Spex-Jobin Yvon). The synchronous spectra (SFS) were recorded in the 240–700 nm excitation range with the emission-excitation offsets ($\Delta\lambda$) in the 10–160 nm range, with a 10 nm step. The slit widths of the excitation and emission monochromators were set at 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 was used for the compensation of the xenon lamp intensity fluctuations. The spectra were corrected

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