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Easy-to-use analytical approach based on ATR–FTIR and chemometrics to identify apple varieties under Protected Designation of Origin (PDO)

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ABSTRACT

Currently, the process of accrediting a certain crop to become a Protected Designation of Origin (PDO) valid one for the fabrication of *Sidra de Asturias* implies the visual inspection of the crop by technicians who ensure that there are only PDO valid varieties. It becomes then necessary a rapid, non-destructive, easy-to-use, cheap, trustable and efficient method to objectively classify the crop in order to detect possible frauds or human unintentional errors in the identification.

Rapid methods for identification of different apple varieties based on Attenuated Total Reflectance (ATR)–Fourier Transform Infrared fingerprint spectroscopy assisted by Linear Discriminant Analysis (LDA) and by Artificial Neural Networks (ANN) have been developed. For each assayed variety, different sections of clean apple skins were selected and scanned from 600 cm^{-1} to 4000 cm^{-1}. Apple data extracted from FTIR spectroscopy were gathered, analyzed and the final 372 reduced spectra were randomly distributed into training data sets (70%) and test data sets (30%). The classification results based on the LDA model gave a higher success rate (95.0%) than the ANN algorithm (85.5%) in the test data sets, although ANN presented a lower incorrect classification rate (1.9%) vs. LDA (5.0%).

The ATR–FTIR technique coupled to chemometric approaches demonstrated to be useful, rapid, cheap and easy-to-use for identifying apple varieties. Success rate is better in LDA than in ANN, although ANN has a lower error rate because of its ability to detect 'unclassifiable' samples. These methods may be a helpful industrial approach to ensure the adequate selection of apple varieties to obtain a perfectly-balanced product under the PDO standards.

1. Introduction

For centuries cider has been the most famous and typical alcoholic drink in the northern region of Spain *Asturias*, becoming one of its most important economic resources (Suárez-Valles and Picinellu-Lobo, 2001). Since the creation of the *Protected Designation of Origin* (PDO) "*Sidra de Asturias*" in the beginning of 21st century (BOE, 2003; BOPA, 2002) only 22 varieties of apple are allowed to be used in "Sidra de Asturias" (Dapena de la Fuente and Blázquez-Noguero, 2009), Table 1. The apple variety used is a key element in the organoleptic characteristics of the final cider. These varieties are classified into eight classes according to their taste and content of phenolic compounds: sour, sweet, sour–bitter, bitter, bitter–semisour, sweet–bitter, semisour and semisour–bitter (BOE, 2003). The quality of the final cider highly depends on the accurate and proper mixture of these different classes to obtain a perfectly-balanced product. Thus, it becomes necessary a rapid, non-destructive, easy-to-use, cheap, trustable and efficient method to objectively classify the crop in order to detect possible frauds or human unintentional errors in the identification.

Currently, the reference technique for identifying Asturian or Basque apples is chromatography (Alonso-Salces et al., 2004; Arias-Abrodo et al., 2010) which also allows determining the degree of ripening (Alonso-Salces et al., 2005), although other techniques such as ^{1}H NMR have also been checked for identifying apple varieties in apple juices or vinegars (Boffo et al., 2009; Del Campo et al., 2006).

However, HPLC and GC have a series of important drawbacks: sample pre-treatment uses to be long and tedious, standards and calibration are required and it is a time-consuming, expensive

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Table 1
Varieties of apples allowed in PDO "Sidra de Asturias". The varieties used in this study appear in bold.

Class	Varieties		
	Durona de Tresali	Blanquita	Limón Montés
Sour	Teórica	San Roqueña	**Raxao**
	Xuanina	Fuentes	
Sweet	**Verdialona**	Ernestina	
Sour–bitter	**Regona**		
Bitter	Clara		
Bitter–semisour	Meana		
Sweet–bitter	**Coloradona**		
Semisour	Carrio	**Solarina**	De la Riega
	Collaos	Perico	Prieta
	Perezosa		
Semisour–bitter	Panquerina		

and destructive technique which is also difficult to implement in an on-line protocol.

Infrared-spectroscopy (IR) arises as a valid alternative for this kind of experiments with a performance comparable to that of HPLC (Liu et al., 2006; Rudnitskaya et al., 2006). Every functional group has a characteristic region of absorption in the IR region according to its general stretching, bending and wagging molecular vibrations; thus, the overall spectrum may be considered as the fingerprint for a given chemical. FT-IR is an extremely fast, cheap, green, simple to use technique with almost no sample pre-treatment and it shows a high selectivity when coupled with chemometric data analysis techniques (Downey, 1996, 1998). These advantages become specially evident when comparing FTIR with other techniques used with the same aim, such as HPLC or NMR (Alonso-Salces et al., 2004; Arias-Abrodo et al., 2010; Boffo et al., 2009). In particular, the mid infrared (MIR) spectroscopy in food analysis is mainly used for qualitative purposes and, concerning specifically apple products, MIR was used to authenticate apple purees (Defernez et al., 1995), classify apple beverages (Andrade et al., 2003; Gestal et al., 2004) and to quantify organic compounds in apple juices (Kelly and Downey, 2005; Leopold et al., 2009, 2011).

The near-infrared (NIR) region includes information about overtones and combinations of molecular vibrations, and it is widely used for qualitative and quantitative analysis in industries, especially in food and agricultural sectors (Downey et al., 2006; Nicolai et al., 2007). Regarding apple products, NIR has demonstrated to be a useful tool to analyze ripeness (Bodria et al., 2004; Menesatti et al., 2009), quality (bruises, breakdown) (Camps et al., 2007; ElMasry et al., 2008), presence of pollutants in the skin (Liu et al., 2007a, pp. 412–418), or to quantify certain compounds (Liu et al., 2007b, pp. 986–989; Ying et al., 2005). Both MIR and NIR techniques have also been co-used to analyze apple juices (Reid et al., 2005).

The composition of the flesh or the pulp of the fruit is well known, and quantification of some their components allows different species to be identified (Alonso-Salces et al., 2004; Del Campo et al., 2006; Ying et al., 2005). The composition of the skin of apples has also been widely studied (Arnous and Meyer, 2008; Guyot et al., 1997; Jakopic et al., 2009; Pinelo et al., 2008) and differences among apples can be easily identified with a simple analysis of the skin (Lees et al., 1995; Li et al., 2004).

Different studies on the phenolic components have shown that they are several times more concentrated in the skin than in the flesh (Guyot et al., 2002; Khanizadeh et al., 2008; Lees et al., 1995), and that the composition is substantially different in both. There are two classes of polyphenols in apple fruit, the non-flavonoids and the flavonoids (Daniel-Kelly and Downey, 2005; Khanizadeh et al., 2008; Le Bourvellec et al., 2011). Non-flavonoids include *hydroxycinnamic acids derivatives* and *dihydrochalcones*. The major constituent of the latter in apples is the *phloridzin*, which presents higher concentrations in the skin than in the flesh (Guyot et al., 2002; Hagen et al., 2006; Khanizadeh et al., 2007, 2008; Le Bourvellec et al., 2011). On the contrary, the *hydroxycinnamic acids* (e.g. *chlorogenic acid*) are mostly located in the flesh (Khanizadeh et al., 2008; Le Bourvellec et al., 2011). *Flavonoids* are polyphenolic phytochemicals with a phenyl benzopyrone structure. They are usually categorized into *flavones*, *isoflavones*, *flavanols*, *flavanones*, *flavonones*, *flavonols* and *flavanonols* (Ravishankar et al., 2013). It has been reported that concentration of flavonoids is higher in the skin than in the flesh (Awad et al., 2000; Hagen et al., 2006). In fact, a certain type of flavanols, anthocyanins, has been reported to be responsible for the coloration of apple skin, with species such as cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside and cyanidin-7-*O*-arabinoside, cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-xyloside, and cyanidin-3-*O*-glucose (Jakopic et al., 2009; Li et al., 2004; Mulabagal et al., 2007). In Table 2, a summary polyphenol composition of apple's skin and flesh is given.

Despite the amount of research carried out on the chemistry of the skin of apples, there are just a few works published on the use of infrared spectroscopy to identify apple varieties, being one of the most interesting the work of He et al. who analyzed apple skins by Vis–NIR to distinguish between three apple varieties (Fuji, Red Delicious and Copefrut Royal Gala) (He et al., 2007).

Under this perspective, the development of easy-to-use analytical tools based on ATR–FTIR and chemometrics to identify the category of a given apple becomes very interesting from two points of view. On the one hand, these procedures would help to avoid fraud and, on the other, these analytical tools may be implemented in the other foodstuffs based on apples production line to automatically separate different classes of apples and raw materials control.

In this paper, new analytical methods based on Attenuated Total Reflectance (ATR)–Fourier Transform Infrared fingerprint spectroscopy assisted by Linear Discriminant Analysis (LDA) or by Artificial Neural Networks (ANN) have been developed for identification of seven apple varieties.

2. Materials and methods

2.1. Chemicals and instrumentation

Methanol was purchased from Prolabo-VWR.

Infrared spectra were taken in a Varian 670-IR FTIR spectrometer (Australia) equipped with a *Golden Gate* horizontal attenuated total reflectance (ATR) accessory. ATR device consisted of a diamond crystal with one internal reflection (crystal area: 1×1 mm). The spectrometer was completely software-controlled by the *Varian Resolutions Pro* software provided by Varian Inc.

2.2. Apples and experimental procedure

Seven different varieties within the twenty-two allowed in the Designation of Origin were selected for this study. They were kindly provided by *the llagar "La Nozala"* (Gijón, Asturias), covering five out of the eight 'taste-types' (BOE, 2003). *Durona de Tresali* (TRE), *Raxao* (RAX) and *Xuanina* (XUA) were chosen as sour apples; *Verdialona* (VER) as sweet; *Regona* (REG) as sour–bitter; *Coloradona* (COL) as sweet–bitter and *Solarina* (SOL) as semisour. Apples were carefully washed with methanol, rinsed with water and dried at room temperature. Due to the heterogeneous visual profile of the apples (Fig. 1), between 16 and 18 different free-of-defects sections of the skin were selected in every apple. Samples were obtained by slicing with a scalpel a thin layer of apple skin, about 1 mm of