Excitation-Emission fluorescence as a tool to assess the presence of grape-must caramel in PDO wine vinegars

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Abstract

A practice in wine vinegar production is the addition of grape-must caramel to correct and unify the final colour of different batches. Although current legislation allows it, the effect in vinegars' quality has not been studied yet and it can become a fraud when it is used to simulate the effect of a longer ageing. Therefore, the aim of this work was to assess multidimensional fluorescence as a cost-effective and fast technique for detecting and quantifying grape-must caramel in vinegars. Different amounts of grape-must caramel and multivariate data analysis, as Parallel Factor Analysis (PARAFAC), N-way partial least squares and partial least squares discrimination and regression (NPLS-DA, PLS-DA and NPLS) were studied. Triangle sensory test was also performed. Results demonstrated the ability of this methodology in the detection and quantification of grape-must caramel (low prediction errors, RMSEP≈0.24) and the effects that grape-must caramel has upon a PDO vinegar's final quality.

Keywords: Wine vinegars, Protected Designation of Origin, Grape-must caramel, Fluorescence, Calibration, Classification.

1. INTRODUCTION

Wine vinegar is the most commonly-used vinegar in both Mediterranean countries and Central Europe. Andalusia is a southern Spanish region traditionally associated with wine growing where three high-quality wine vinegars have been protected under a legal framework called Protected Designation of Origin (PDO): *Vinagre de Jerez*, *Vinagre de Montilla-Moriles*, and *Vinagre de Condado de Huelva* PDOs (Council Regulation (EC) No 510/2006). These high-quality PDO wine vinegars are made from the corresponding protected wines, endowing each vinegar with singular and specific characteristics. All of the PDO regulations require an ageing period in wooden butts and during this ageing period an important number of physicochemical changes take place. These changes are what give the vinegars their unique organoleptic properties and sensory quality (Morales, Tesfaye, García-Parrilla, Casas, & Troncoso, 2002). *Vinagre de Jerez* and *Vinagre de Montilla-Moriles* PDOs have established the same categories regarding sweetness, time and method of ageing (the *criaderas and solera* and *añada* system*)*: *Pedro Ximenez* category (sweet category), *Crianza* (aged in wood for at least 6 months), *Reserva* (with a minimum ageing time of 2 years.) and *Gran Reserva* (aged for 10 or more years). During ageing, the flavours of the barrel are absorbed by the vinegar and therefore, their quality increases. This fact raises the final market price, thus making them more vulnerable to frauds (Callejón et al., 2012). This means that PDO wine vinegar quality assurance and authentication are highly important issues.

Authenticating and characterising PDO-labelled vinegars with the aim of assuring their quality, is important for protecting the consumer against being sold an inferior quality or counterfeit product (Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016; Karoui & De Baerdemaeker, 2007). The unfair activities related to high-quality wine vinegars that bear a PDO label range from incorrect labelling to production outside PDO regulations or even to

adding substances prohibited by the regulations. One of the substances added to the vinegars is grape-must caramel.

Grape-must caramel, also called 'grape syrup', is a sweetening and colouring agent obtained after boiling the grape must which is very rich in sugars and is brown in colour (Ortega-Heras & González-Sanjosé, 2009). It is commonly added to some Spanish wines in order to obtain special sweet wines. The addition of grape-must caramel to Spanish PDO wine vinegars is an allowed practice performed to unify the final colour of vinegars of different batches. The amounts required for this purpose are low and they should not affect the organoleptic characteristics of the final products. However, due to the fact that a maximum limit of addition has not yet been established, this could lead to some adulterations with the aim of modifying some of the characteristics of the final wine vinegar.

During ageing the colour of wine vinegar changes from amber to mahogany. The content and concentration of polyphenols, tannins and anthocyanins as well as an oxidation process are the main factors involved in the vinegar's darkening. Many of these compounds are also present in grape-must caramel, making determination of the presence of grape-must caramel in vinegars a difficult issue. In this context, the addition of grape-must caramel to the final wine vinegars could be used to simulate the effect of a greater wood ageing in wine vinegars. It has been demonstrated that the addition of grape-must caramel to a wine vinegar produces significant changes in its composition and final characteristics with a large increase in both brown tonalities and sweetness (Ortega-Heras & González-Sanjosé, 2009). Thus, the addition of grape-must caramel to a vinegar could change its organoleptic characteristics, the final product being different from the raw one. All of these facts illustrate the need for an analytical tool to determine and monitor the addition of grape-must caramel to PDO-protected wine vinegars.

In recent years, interest has been growing in developing rapid, inexpensive, non-destructive and direct methodologies based on non-targeted techniques for food characterisation. In this context, today excitation-emission fluorescence spectroscopy has an important role. Among the advantages of fluorescence spectroscopy is the enhanced selectivity when compared to other spectroscopic techniques; its high sensitivity to a wide range of potential analytes and an easy – or even unnecessary – sample pre-treatment (Sayago, García-Gonzalez, Morales, & Aparicio, 2007). Fluorescence spectroscopy has been applied as a competitive, high sensitivity, fast and non-destructive technique in food analysis (Karoui & Blecker, 2011). In a previous study (Ríos-Reina et al., 2017) this methodology demonstrated its usefulness for characterising and classifying PDO wine vinegars

Measuring the emission spectra at different excitation wavelengths results in a three-dimensional Excitation-Emission Matrix (EEM) array, which contains information unique to each measured sample. Nowadays, the instrumental improvements and the availability of software specially designed to extract information contained in spectra has enabled the use of EEM in combination with chemometric methods in order to characterize and detect adulteration in different matrices, such as different food products and beverages (Azcarate, Teglia, Karp, Camiña, & Goicoechea, 2017; Casale et al., 2018; Elcoroaristizabal et al., 2016; Öztürk, Ankan, & Özdemir, 2010; Sayago et al., 2007), as well as in many other matrices (Heidari, Hemmateenejad, Yousefinejad, & Moosavi-Movahedi, 2018; L. Zhu et al., 2016). The analytical information contained in fluorescence spectra can be extracted in order better to interpret it using various multivariate analysis techniques that relate several analytical variables to the analytes' properties. One appropriate multiway method for extracting and interpreting the maximum information possible from this matrix is PARAllel FACtor Analysis (PARAFAC). It has been applied in

order to break fluorescence EEMs down into different independent groups of fluorophores, as well as their relative concentration (scores) in each sample (Bro, 1997). The information provided by the resolved fluorophores has been successfully applied in food quality control since it can reveal clearer insights into the relationships between the intrinsic food properties and the quality of the product. Moreover, the extracted fluorophores could be used for a classification approach by discriminant analytical methods such as partial least squares-discriminant analysis (PLS-DA). In addition, the EEM array could also be studied directly with the use of multivariate calibration methods such as N-way partial least squares (N-PLS) that have also made it possible to relate instrument responses that consist of several variables to a chemical or physical property of a sample, as well as with multiway discrimination analysis such as NPLS-DA.

The aim of this study was to assess the potential of excitation-emission fluorescence spectroscopy combined with three-way methods of analysis (PARAFAC and multiway N-PLS regression) and discriminant analysis (PLS-DA and NPLS-DA) to detect and classify the different additions of grape-must caramel in PDO wine vinegars. It is the first time that a methodology for the determination of gape-must caramel has been established. Different amounts of grape-must caramel were added to PDO wine vinegars that were grape-must caramel free in their raw composition. In addition, commercial PDO wine vinegars (that actually could have some added grape-must caramel) were also analysed to test the models and to determine their amount of caramel. For this purpose, Parallel Factor analysis (PARAFAC) was applied for pre-processing the three-dimensional arrays in order to study the potential fluorophores related to this addition. Multivariate data analysis (PCA, PLS-DA) was then performed in order to differentiate and classify samples that had or did not have grape-must caramel in different concentrations. Consequently, the discrimination results were compared to those obtained by a multiway partial least-squares discrimination

analysis (NPLS-DA). Finally, regression models were developed in order to attempt to predict and quantify the level of grape-must addition by relating the PARAFAC components to the chromatographic compounds detected, or by using the EEM array by N-PLS regression method. Additionally, a sensory test was developed to evaluate the influence of added grape-must caramel on the organoleptic properties of the PDO wine vinegars and to propose a possible addition limit that does not affect or modify their unique final organoleptic properties.

2. MATERIALS AND METHODS

2.1. Samples

Wine vinegar samples from two Spanish PDOs (*Vinagre de Jerez* and *Vinagre de Montilla-Moriles*) were analysed in this study: 16 commercial wine vinegars from the *Crianza* category (CR), aged for 6 months to 2 years (10 from *Vinagre de Jerez* PDO and 6 from *Vinagre de Montilla-Moriles* PDO) and 18 commercial wine vinegars from the *Reserva* category (RE), aged from 2 to 10 years (13 from *Vinagre de Jerez* PDO and 5 from *Vinagre de Montilla-Moriles* PDO). These samples were collected working in compliance with the Regulatory Councils and were grouped in this study as the Unmodified group. Finally, 2 caramel-free samples of both *Crianza* and *Reserva* (one from each PDO) were collected from the wineries and included in the study as Control samples. More information and codification of samples is shown in Table 1.

2.2. Reagents and Chemicals

The grape-must caramel (also named colourant caramel MO-7) used was supplied by SECNA S.A. (Valencia, Spain), with identification number CEE: E - 150 d. Water was obtained from Milli-Q purification system (Millipore, USA). Analytical-quality acetic acid and methanol were supplied by Merck (Darmstadt, Germany). 5-Hydroxymethylfurfural (5-

125 HMF) according to the standard OIV (2009) method was purchased from Sigma-Aldrich
126 (Madrid, Spain).

127 2.3. Grape-must caramel addition

128 First, thirteen different amounts of a dilution of grape-must caramel (10/100 v/v) were
129 added to 10 mL of vinegar: 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, and 250 µL.
130 The amounts added were selected by examining the total range of colours of the
131 commercial wine vinegars. These samples were grouped into a class called Modified. The
132 vinegars selected as a matrix of these different additions were the *Crianza* and *Reserva*
133 vinegars without caramel in their composition collected directly from the winery and
134 belonging to both PDOs were designated as the Control samples. In table 1, therefore,
135 these samples appear in the Modified-control matrix group. Moreover, among these
136 samples made, five, with intermediate concentrations of grape-must caramel (20, 40, 75,
137 125, 175 µL), were used as the test set for assessing the robustness of the regression
138 models. These additions are expressed in Table 1 as % v/v.

139 In addition, and in order to include more samples in the models, the same procedure was
140 performed using a commercial *Crianza*-category wine vinegar from each PDO (also
141 grouped as Modified samples) by making 8 points of the above mentioned (group of
142 samples named in the study as Modified-Commercial matrix). Two replicates per level
143 were performed. A total of six curves were obtained by varying the matrix where the
144 grape-must caramel was added: 4 *Crianza* (two control and two commercial matrices) and
145 2 *Reserva* wine vinegars (control matrices). This information is more easily shown
146 schematically in Table 1.

147 Finally, the same calibration levels were performed in a hydroacetic matrix at 6% in order
148 to study the pure grape-must caramel. A schema and some photos of these curves are
149 shown in Supplementary Fig 1.

150 2.4. Fluorescence analysis

151 Fluorescence measurements were recorded using a Varian Cary-Eclipse fluorescence
152 spectrophotometer (Varian Iberica, Madrid, Spain), equipped with two Czerny-Turner
153 monochromators, and a Xenon discharge lamp pulsed at 80 Hz with a half peak height of
154 2 ms (peak power equivalent to 75 kW). A high-performance R298 photomultiplier tube
155 detector was used for collecting the fluorescence spectra. Wine vinegar samples were
156 analysed directly without sample pre-treatment by pipetting them into 3.5 mL quartz
157 cuvettes before measurement. 1-cm path length standard quartz cells (Hellma Analytics,
158 Müllheim, Germany) were used to perform the measurements in a Peltier thermostatic
159 cuvette holder (25.00 ± 0.05 °C). The spectrometer was interfaced to a computer with
160 Cary-Eclipse software for spectral acquisition and exportation.

161 The fluorescence Excitation-Emission Matrices (EEMs) were obtained by varying the
162 excitation wavelength (λ_{ex}) ranging between 250 and 650 nm (every 5 nm), and recording
163 the emission spectra (λ_{em}) from 300 to 700 nm (every 4 nm). For these measurements,
164 excitation and emission slits were both set at 5 nm, and the scan rate was fixed to 1200
165 nm min^{-1}. The system was wavelength-calibrated every day by means of the water Raman
166 peak to account for a possible instrument wavelength drift. EEMs were recorded in
167 triplicate for each wine vinegar type and each level of the calibration and pre-processed in
168 order to avoid noisy and non-informative areas by selecting shorter spectral ranges (λ_{ex}
169 from 300 to 650 nm, and λ_{em} from 300 to 700 nm).

170 2.5. High-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed using a LaChrom® WWR-Hitachi (Barcelona, Spain) liquid chromatograph with a quaternary L-7100 pump connected to an L-7455 diode array detector (DAD). The column was a Luna C18, 5 µm, 250 x 4.6 mm and a guard precolumn of 4.0 x 3.0mm from Analytical Phenomenex (Torrance, CA, USA). Detection was performed at 280 nm. The injection of the samples (10) µL was performed using an L-2200 autosampler and the separation was obtained at a flow rate of 1.2 mL min^{-1} with an isocratic elution. The analysis takes less than five minutes.

The mobile phase consisted of 80% water, 18% methanol and 2% acetic acid. Previously filtered through a 0.45 µm PTFE membrane filter (Merck, Darmstadt, Germany), the samples were analysed in duplicate. Quantification of 5-HMF was performed according to Elcoroaristizabal et al., 2016, by using an external calibration curve in the range between 5 and 80 ppm. A calibration curve at 6 levels with two replicates per level was built using the least-squares method. The response of the 5-HMF standard was linear within the concentration range tested, with a determination coefficient of R^2 = 0.997. Standard solutions were prepared using a hydro-acetic matrix (6% v/v).

2.6. Sensory analysis

An olfactory and taste analysis was carried out. The expert sensory panel comprised eight tasters (six females and two male), all belonging to our laboratory and with extensive experience in wine vinegar sensory analysis. For the olfactory test, fifteen millilitres of each sample were presented in coded opaque glasses to mask the colour while following the protocol for vinegars established by Tesfaye et al., 2010. For the gustative test, a drop of each sample was placed in a coffee spoon.

Firstly, an ascending order test was performed to delimit the correct concentration range of grape-must caramel to study and to familiarize panellists with the odour of the samples.

195 Panellists were asked to indicate in which glass and spoon they perceived any change of
196 odour or flavour. The starting point was the CR control without any caramel. Secondly,
197 triangular tests (IS0 4120 - 1983) were performed to ascertain whether the panellists were
198 capable of discriminating caramel-free samples from those vinegars with added grape-
199 must caramel. Moreover, triangle tests were also performed to assess the capability of
200 discriminating some *Reserva* commercial wine vinegars from the modified wine vinegars
201 from each PDO.

202 2.7. Software and data analysis

203 2.7.1. Pre-processing of spectra and PARAFAC analysis

204 EEMs data were pre-processed in order to correct Rayleigh and Raman scattering
205 (Elcoroaristizabal, Bro, García, & Alonso, 2015) by removing and replacing the scattering
206 areas with interpolated values by using the FLUCUT function included in the
207 PLS_Toolbox. The corrected EEM matrices underwent PARAlell FACtor analysis
208 (PARAFAC) (Bro, 1998) in order to extract the relevant information and to develop models
209 for differentiating authentic samples from those with added grape-must caramel. This
210 methodology is not described here due to having been described in a previous study
211 (Ríos-Reina et al., 2017). The number of factors for each model was determined by using
212 the CORe CONsistency DIAgnostic test (COR-CONDIA) (Bro & Kiers, 2003), the model
213 percentage of explained variance and by visual inspection of the recovered spectral
214 profiles and residuals. Non-negative constraints for all modes were applied.

215 2.7.2. Exploratory and classification analysis

216 2.7.2.1. PCA and PLS-DA on the PARAFAC factors

217 In order to perform a first screening of samples and to reflect the sample distribution in
218 latent space, principal component analysis (PCA) was applied to the scores of the

PARAFAC factors obtained. Moreover, classification accuracy was calculated by means of Partial Least Squares-Discriminant Analysis (PLS-DA). This algorithm was used to build classification models for discriminating the Unmodified (commercial) wine vinegar samples from the Modified samples, that is, those CR and RE with the addition of grape-must caramel and the control ones, in order to test the ability of the methodology to discriminate between the presence or absence of grape-must caramel at different levels. Furthermore, the data was autoscaled and samples were randomly divided into the training set (comprising 75% of samples) that was used for data modelling and internal validation by means of a venetian blinds cross-validation, and a test or prediction set used for evaluating the discriminative power of the models (external validation).

2.7.2.2. N-PLS discriminant analysis (NPLS-DA)

NPLS-DA was applied to the three-dimensional array, which was prior multiway centred, in order to compare the classification results of a multiway analysis to the previous one-way approach (i.e. PLS-DA classification by the use of the PARAFAC factors). NPLS-DA is an extension of PLS, used in the case of data in three-dimensional arrays. Thus, the NPLS-DA consists of applying the N-PLS algorithm to classification, predicting the membership of a sample to a qualitative group defined as a preliminary (Vigneau, Qannari, Jaillais, Mazerolles, & Bertrand, 2006). In essence, N-PLS for discriminant analysis is the same as for calibration purposes. Discrimination quality was obtained by comparing the predicted groups to the real groups and is shown as the percentage of correct classification. The data was again autoscaled and randomly divided again into two sample sets, as had been the case with the PLS-DA model: the training set (comprising 75% of the samples) that was used for calibration and internal validation of the models by means of a venetian blinds cross-validation, and a test set used for evaluating the discriminative power of the models employed as an external validation.

2.7.3. Correlation of wine vinegars EEM spectra with grape-must caramel

Regression models based on PARAFAC and N-PLS algorithms were compared. On the one hand, the area of the compounds detected by HPLC as well as the % v/v of grape must-caramel were correlated to the extracted PARAFAC components. On the other hand, a multiway linear regression analysis, called N-way partial least squares (N-PLS), was built using the EEM data which was multiway centred in order to determine the presence of grape-must caramel in the commercial PDO wine vinegars by the fluorescence landscapes kept as three-way array. Regression models were evaluated using the figures of merit: Root Mean Square Error of calibration, cross-validation and prediction (RMSEC, RMSECV and RMSEP) as a term to indicate the prediction error of the model, and the coefficient of determination (R^2). R^2, generally used for evaluating model quality, is the correlation coefficient between the predicted and actual/measured grape-must caramel. RMSEC is used to compare quality of the results provided in the calibrations and it is expressed as a percentage (in both calibration and prediction), taking into account the response range in its calculation (Sáiz-Abajo, González-Sáiz, & Pizarro, 2006). The data was multiway centred across the first mode (i.e. sample mode) and divided into two sets, train and test. Venetian blinds was applied by means of cross validation.

2.7.4. Software

EEM data modelling and chemometric analyses were performed by using the PLS_Toolbox 7.9.5 (Eigenvector Research Inc., Wenatchee, WA) working under Matlab v.8.5.0 environment (The Mathworks Inc., Natick, MA).

3. RESULTS AND DISCUSSION

3.1. Visual assessment of fluorescence landscapes

Fig. 1 shows, in the left side (a), an example of the fluorescence landscapes in the form of contour plots (after removing and replacing the scattering areas) of different levels of the calibration curve made with the *Crianza* Control wine vinegars as matrix (those without caramel obtained from the wineries) from both PDOs, including also the *Reserva* Control wine vinegars on the far right of the figure (Fig. 1a). Moreover, the calibration curve produced with the hydroacetic matrix is also shown at the left bottom of the figure (Fig. 1c).

As can be observed, a visual assessment of the fluorescence landscapes indicated a similar profile for vinegars of both PDOs, with fluorophores overlapping in both excitation and emission dimensions, together with some differences due to the addition of grape-must caramel. Thus, the fluorescence profiles of the *Crianza* vinegars without grape-must caramel (first samples in the rows) showed a common maximum peak around 370/450 nm for both excitation/emission wavelengths ($\lambda_{ex}/\lambda_{em}$), although in the *Reserva* control samples (last samples in the rows) the maximum peaks appeared at slightly higher wavelengths, around 370–470 nm of λ_{ex} and 470–550 nm of λ_{em}. These features were similar to those observed in a previous work studying PDO wine vinegars (Ríos-Reina et al., 2017).

Additionally, the visual assessment of the EEM landscapes with and without the addition of grape-must caramel allows an a *priori* confirmation of differences between samples by looking at the areas where the potential compounds appeared. Thus, for example, the peak at 370/450 nm ($\lambda_{ex}/\lambda_{em}$) tended to disappear as more grape-must caramel was added, giving way to the appearance of a second peak around 550/570 nm of excitation and emission wavelength, respectively. It should be also noticed that, as the commercial samples are able to present some grape-must caramel, some of the analyzed in this study already showed this trend. Moreover, another important feature was that as more grape-

must caramel was added to the vinegar, EEM intensity decreased. This behaviour was also observed as being PDO-independent – even in the hydroacetic matrix analysed (Fig. 1c). In fact, the hydroacetic samples with different amounts of grape-must caramel showed similar trends, also being similar to the vinegar samples due to the fact that it should be considered that grape-must caramel has many grape-derived compounds, such as wine vinegars. However, the excitation/emission wavelengths were not exactly the same, due to the relevant phenomena related to the nature of the food and its molecular environment, both of which influence the fluorescence signal. This is commonly called the matrix effect (Azcarate et al., 2017). All of these results partially demonstrated that excitation-emission fluorescence was able to detect those samples whose colour was modified by the addition of grape-must caramel.

3.2. Decomposition of the spectral data in the potential fluorophores by using PARAFAC

In order to observe and evaluate the pure spectra of fluorophores related to the addition of grape-must to wine vinegars, an adequate multiway method for pre-processing the three-dimensional array was carried out. Thus, the EEM landscapes of all of the samples under study (the Modified and the Unmodified samples of both categories and both PDOs) were decomposed into the main fluorescence contributions by using PARAFAC analysis. The best PARAFAC model built for each PDO was obtained with five factors, giving final reliable models that explain more than 99% of the variance and with a core consistency over zero. Fig. 1 also shows in the right side the PARAFAC loadings (excitation/emission profiles) of each main fluorophore obtained for both PDOs (Fig. 1b) and hydroacetic matrix with different amounts of grape-must caramel (Fig. 1d). A great similarity of the spectral profiles acquired for both PDOs (*Vinagre de Jerez* in discontinuous lines and *Vinagre de Montilla-Moriles* in continuous lines) could be observed. This fact suggests that these

317 fluorescence fingerprints could be useful for addressing the problem under study, as it
318 shown to be PDO-independent. Similar results were obtained by Elcoroaristizabal et al.,
319 (2016) in the study of different types of *Cava* in which a great similarity of the spectral
320 profiles was obtained independently of the *Cava* analysed.

321 The fluorescent loading patterns of the modelled factors in the PDO samples can be
322 matched to fluorophores described in the literature. The first factor (F1, blue in Fig. 1b)
323 therefore, has a similar profile for the two PDOs under study with excitation and emission
324 maxima centred around 380 nm and 450 nm, respectively. This factor also appeared in the
325 previous study (Ríos-Reina et al., 2017) and was related to the cumarins, tannins,
326 phenols, flavonols that are naturally present in wine.

327 The second factor (F2, red in Fig. 1b) is a peak centred at 400-430nm of excitation and
328 500-520 nm of emission. This fluorophore could be matched with Maillard compounds
329 according to Zhu, Ji, Eum, & Zude (2009) and Ríos-Reina, et al. (2017), formed in
330 vinegars during ageing (García Parrilla, Heredia, & Troncoso, 1999). According to the
331 literature, within these compounds, 5-HMF is one that has been shown to have a high
332 correlation to these wavelengths (Callejón et al., 2012). Grape-must caramel also has high
333 amounts of this compound. In this regard, it is important to emphasize that each
334 PARAFAC factor probably corresponds to a related fluorescent molecule group, and not
335 necessarily to a single fluorescent molecule and for that reason, this factor could be
336 matched with different compounds, although from a similar family.

337 The third factor (F3, yellow in Fig. 1b) shows an excitation maximum around 470 and the
338 emission one at 550 nm for both PDOs although for *Vinagre de Jerez* this factor shows a
339 shoulder at 350 nm of excitation that could be due to differences in the composition
340 between the two PDOs. According to the literature (Airado-Rodríguez, Durán-Merás,
341 Galeano-Díaz, & Wold, 2011) and our previous knowledge (Ríos-Reina et al., 2017), the

common parts of this factor appeared to be related to vitamin B2 and its principal forms such as Riboflavin, Flavin mononucleotide (FMN), and Flavin adenine dinucleotide (FAD)

The fourth factor (F4, purple in Fig. 1b) has excitation and emission maxima between 320-340nm and 400-420 nm, respectively. In this case, the *Vinagre de Montilla-Moriles* factor shows a small shoulder at 450 nm of emission, different to the other PDO. According to the results presented in the literature, excitation/emission wavelengths around 330/420 nm have been related to phenolic acids and phenolic aldehydes, as well as oxidation and Maillard reaction products (present due to browning processes and oxidative mechanisms taking place during ageing and storage) (Airado-Rodríguez et al., 2011; Azcarate et al., 2015; Callejón et al., 2012; Dufour, Letort, Laguet, Lebecque, & Serra, 2006; Elcoroaristizabal et al., 2016; Sádecká & Tóthová, 2007).

Finally, the fifth factor (F5, green in Fig. 1b) shows a peak centred at 550 nm of excitation, with a shoulder at 400 nm in both PDOs, and an emission maximum around 600-630 nm. This has not previously been associated to any fluorophore. However, this factor was similar to the one obtained in the previous work (Ríos-Reina et al., 2017), which showed a relationship to *Pedro Ximenez* wine vinegars. Consequently, higher mean values of this factor were obtained for samples belonging to this category. The sweet category is produced by adding raisined *Pedro Ximenez* grape must or adding *Pedro Ximenez* wine to the vinegar. Therefore, the concentration of grape-must should be higher in these sweet vinegars than in the *Crianza* or *Reserva* ones. For this reason, the presence of this factor in our samples also appeared to be related to the addition of grape-must caramel, it being, therefore, a relevant factor to take into account in this study.

As mentioned earlier, it is relevant to consider the phenomena related to the nature of the food that will influence the fluorescence signal. These phenomena are related to the inherent fluorophores' concentration and their environment. Therefore, a specific

fluorophore studied in different foods can present different spectral signals (Azcarate et al., 2017). In fact, adding grape-must caramel changes the environment of the natural wine vinegar fluorophores and so could have the ability to modify the signal, as can also be observed in the 5-factor PARAFAC model of the hydroacetic matrix with only grape-must caramel in its composition (Fig. 1d). Thus, the PARAFAC model built with the curve of grape-must caramel in a hydroacetic matrix (Fig. 1d), shows similar fluorophores as in the vinegar matrix, but some of them are displaced. In spite of this, the fifth factor (F5 in green, Figure 1.d) matched perfectly in terms of excitation/emission wavelengths with the fifth factor of the PARAFAC models developed with the PDO wine vinegars, which appeared to have a strong relationship with the presence of grape-must caramel.

In fact, only the scores of the fifth PARAFAC factor (F5) extracted from the hydroacetic curve showed an increase in the case of added grape-must caramel, appearing to follow a logarithmic kinetic (Supplementary Fig. 3). Hence, the scores of the F5 described a logarithmic kinetics equation as follows:

$$Y = m\mathrm{Ln}(Y_0)+b;$$

where Y is the score value of F5 (a.u.), m is the slope, Y_0 is the initial value of F5 score (a.u.), and b the intercept. Thus, the logarithmic kinetic obtained with the fifth PARAFAC factor, which is shown in Supplementary Fig. 3), was $Y=42.538\mathrm{Ln}(Y_0)+148.15$.

3.3. Exploratory analysis

A principal component model was developed with all of the Modified and Unmodified samples for each PDO by using the extracted PARAFAC factors in order to explore the data and to detect grouping and outliers in each PDO. The scores and loadings plots are shown in Fig. 2. In general, a separation of both groups (modified and unmodified) could

be observed in the two PCA models for both PDOs, which means that the methodology appeared to be able to detect the addition of grape-must caramel.

In the case of the *Vinagre de Montilla-Moriles* PCA model (Fig. 2a), the first component (PC1) is the main factor in the separation, explaining 69.30% of the original variance, showing a good separation of the groups, the modified samples being located on the negative side of PC1 and the unmodified on the positive side. However, it was also observed that three unmodified samples (i.e. commercial samples) were grouped closely to the modified ones, especially two RE samples located next to the samples containing the most added grape-must caramel. These results suggest that these two RE samples could have a higher amount of grape-must caramel in their composition than the other commercial samples, something that could change the raw organoleptic characteristics by binding the effect of some compounds related to ageing; or it could even be a case of unfair practice, these RE samples in fact being CR vinegars with added grape-must caramel in order for them to resemble the colour of an RE.

With regard to the Modified samples, those with the lowest amounts of grape-must caramel (lower than 0.1% v/v) were located near to some commercial samples. Thus, a commercial *Crianza* sample was observed located very close to a Modified wine vinegar in the scores plot, this modified sample being a *Crianza* Control vinegar containing 0.05% grape-must caramel. These results showed that some commercial samples could have a very low amount of grape-must caramel in their final composition. In terms of the loadings plot, and due to its position on the plot, the fifth factor once again appeared to be the greatest factor regarding the presence of grape-must caramel, followed by F4.

With regards to the PCA model of *Vinagre de Jerez* (Fig. 2b), the separation in this particular case appeared to be more related to PC3. Thus, observing the scores plot of PC1 vs PC3, modified samples were located on the negative side of PC3, although once

again, a few unmodified samples (some CR and RE commercial samples) were not properly separated from the modified ones in this model. As before, this placement could be explained by a greater amount of grape-must caramel in their composition than the rest of samples, thus affecting the composition by binding some relevant compounds. These wrongly-placed RE commercial samples therefore appeared to have more similarities according to their scores with the RE samples modified with 1-2.5% v/v of grape must caramel, as well as the fact that the aforementioned wrongly-placed CR commercial samples appeared to be more similar to the CR samples modified with 1.5-2% v/v of grape-must caramel.

The separation of both groups of samples was again explained by the F5, as could be observed in the loadings plot. However, when observing the loadings plot, F4 and F1 also appeared to play an important role in this separation. This partially agrees with the results mentioned above (Section 3.1) in which F4 was related to Maillard reaction products that could be derived from the grape-must caramel.

3.4. Classification analysis of modified (by adding grape-must caramel) and unmodified samples (commercial wine vinegars)

Once the ability of the multidimensional fluorescence spectroscopy in distinguishing the presence of grape-must caramel at different levels was demonstrated, the next step was to gain an insight into this differentiation and to determine if the extracted PARAFAC fluorophores allows the classification of samples according to the modification of vinegars with grape-must caramel. To this end, PLSDA classification models were performed using the extracted PARAFAC factors. Moreover, in order to consider the contribution of multiple effects and not only the most relevant information (PARAFAC factors), NPLS-DA classification models were also performed, taking the multiway arrays (EEMs) into consideration. Both classification models were therefore studied and compared in the

following section. Prior to the classification analysis, the data set was randomly partitioned into two sets, train and test, and all of the datasets were mean-centred before developing the models.

3.4.1. PLS-DA classification between modified and unmodified wine vinegars using the extracted PARAFAC factors.

Two PLS-DA models were developed according to each PDO including samples from the two groups in the train and test sets. The *Vinagre de Jerez* PLS-DA model was obtained using 4 latent variables (LVs), which explained 99.75% of total variance, while the PLS-DA model of *Vinagre de Montilla-Moriles* was obtained using 3 LVs and explained 96.83% of total variance. Table 2 shows the PLS-DA classification results expressed as the percentage of correct classification and the number of samples misclassified for each class. Additionally, the statistical performance parameters of the classification models (i.e. sensitivity, specificity and classification error of calibration (CAL), cross-validation (CV) and prediction (PRED)) are shown in Supplementary Table 1. Correct classification rates of 100% were obtained for both Modified and Unmodified groups in the training set for each PDO. In this way it was observed that the models were able to classify the unmodified samples, where both CR and RE commercial samples are grouped, from those modified with the addition of grape-must caramel. To test the models, those commercial samples that were not well-located on the previous exploratory models were purposely included in the prediction sets, together with other unmodified and modified samples in order not to disturb the model's calibration. The classification results enabled the results observed by the previous PCA models to be confirmed, since the seven misclassified samples were those that behaved differently to the rest of commercial PDO wine vinegars.

Moreover, the classification results showed that a 100% correct classification was achieved for all of the modified samples for the prediction set, confirming the good

predictive ability of the classification models developed and, hence, multidimensional fluorescence spectroscopy's ability to detect the addition of grape-must caramel to wine vinegars.

Furthermore, the possibility of taking both PDOs into account together was tested. Table 2 shows that the PLS-DA model obtained with 5 latent variables and 99.64% of total variance explained, again classified the same seven unmodified samples as modified wine vinegars. However, in spite of the fluorescent components appearing to be very similar in both PDOs, when a classification is performed by including both PDOs together, the percentage of correct sample classification was lower than in the separated models.

3.4.2. NPLS-DA classification between modified and unmodified wine vinegars using the three-dimensional arrays EEM.

Once again it should be emphasised that each factor probably does not necessarily correspond to a single fluorescent molecule (Elcoroaristizabal et al., 2016). It is, therefore, possible that different factors need to contribute in order to explain a group of compounds. For this reason, a multiway classification approach was studied. In this case the three-dimensional arrays (EEMs) were used, NPLS-DA was performed and their results were compared to those obtained by PLS-DA with the PARAFAC factors. NPLS-DA classification results are also shown in Table 2. In addition, the statistical performance parameters of the NPLS-DA classification models are shown in Supplementary Table 1. It can be seen that a highly discriminant NPLS-DA model was obtained by using three PLS factors for both *Vinagre de Jerez* and *Vinagre de Montilla-Moriles* models. Here, and similar to the previous PLS-DA results, six commercial samples (three of *Vinagre de Jerez* and three of *Vinagre de Montilla-Moriles*) were classified as being modified with grape-must caramel. Moreover, the number of latent variables needed to explain the classification, the percentage of total variance explained and the samples misclassified

(Table 2), as well as sensitivity and specificity (Supplementary Table 1), were almost the same for the previously-discussed PLS-DA and the NPLS-DA models. As a result, both approaches could be good options to consider. This could demonstrate that the fluorophores extracted by PARAFAC were sufficient to explain the grape-must caramel effect. However, although the multiway classification approach is faster and easier to develop than undertaking PARAFAC and a PLS-DA, it provides less information with respect to the fluorophores involved.

With regard to the model considering both PDOs together and obtained by 3 LVs, better classification rates could be observed (higher percentage of correct classification and less latent variables needed) for NPLS-DA than for the model obtained by PLS-DA and PARAFAC factors, although, once again, the same seven commercial samples were misclassified. This could be explained by the fact that in the multiway discrimination methodology the whole fluorescence matrix is considered. This enables all of the fluorophores related to caramel and to the effect of its environment to be modulated, as well as being able to modulate the interferences.

3.5. Correlation between the additions of grape-must caramel and EEMs.

3.5.1 Univariate calibration - HPLC analysis

After confirming the changes in vinegar components observed in the EEMs with the addition of grape-must caramel, and in order to ascertain the specific compound concentrations which increase or change with such an addition, a chromatographic analysis was performed including the modified and unmodified samples, as well as the hydroacetic solution (Fig. 3). In all of these analyses, three compounds were principally observed to increase when grape-must caramel was added with the following elution order (Fig. 3a): 2.3, 2.7 and 4.2 min of retention time. The first two compounds were unidentified, whereas the last was identified by its corresponding standard as 5-

515 hydroxymethylfurfural (5-HMF). The 5-HMF and the compound termed as unknown 2,
516 (retention time at 2.7 min), presented in all of the samples, while unknown 1 (retention
517 time at 2.3 min) did not present in the wine vinegar matrices which had no grape-must
518 caramel in their raw composition (Control samples).

519 Some studies in the literature show that grape-must caramel has a high amount of
520 furfural-related compounds, including which 5-HMF (Ortega-Heras & González-Sanjosé,
521 2009). 5-HMF is a furanic compound formed during Maillard reactions or by direct
522 dehydration of sugars under acidic conditions (caramelisation) during thermal treatments
523 applied to foods(Capuano & Fogliano, 2011). Hence, its concentration should be high in
524 grape-must caramel. However, as can be observed in the calibration curves of the areas
525 of the three compounds and in the % of grape-must caramel (Fig. 3b), the compound that
526 presented the highest slope was the one named unknown2, and not, as expected, 5-HMF.
527 This could be explained by the fact that other compounds have been also determined in
528 the grape-must caramel and cooked musts, such as melanoidins, caramels (formed by
529 non-enzymatic browning reactions) and other furfurals (Ortega-Heras & González-
530 Sanjosé, 2009; Palacios, Valcarcel, Caro, & Perez, 2002), that could be related to the
531 unknown peaks detected. However, the structure of melanoidins is poorly defined and is
532 not isolated and characterised, making it difficult to identify them.

533 Regarding the commercial wine vinegars under study, especially those samples
534 misclassified as Modified samples which were expected to have a greater amount of
535 grape-must caramel in their composition, the chromatographic results agreed with the
536 fluorescence patterns. Hence, these samples showed higher areas of the two unknown
537 compounds and 5-HMF (i.e. three times more) than the rest of CR and RE commercial
538 wine vinegars.

539 ### 3.5.2 Multiway calibration

In spite of the promising results shown in the previous section, as grape-must caramel is a mixture of compounds and wine vinegar is another complex matrix of compounds, when a univariate calibration was developed with PARAFAC components extracted from the wine vinegar matrix, and not with the hydroacetic matrix, in this case satisfactory results were not achieved. This could be explained by the fact that in order to make correct predictions with the univariate model, the signal of the test samples can only vary due to the analyte, so the contribution of the other species must be the same as what has been modelled. If the contribution of these other species varies (because their concentration varies) or if there is some new interfering signal, the prediction will be biased. The advantage of a multiway calibration over the calibration line is that it allows selective information to be obtained from non-selective instrumental responses (that is, in the presence of interferences), thus enabling the determination of the concentration of various components in complex samples (Olivieri, 2014) to be determined. By using multiway calibration, it has been demonstrated that considerably more complex analytical problems can be solved and predictions are possible – even in the presence of unexpected spectral interferences, i.e., sample constituents not considered in the calibration phase (Arancibia, Damiani, Escandar, Ibañez, & Olivieri, 2012; Bro, 1998; Christensen, Becker, & Frederiksen, 2005; Olivieri, 2014; Olivieri & Escandar, 2014).

For this reason, a multiway calibration method such as N-PLS that considers the entire EEM matrix was studied (Fig. 4). The N-PLS calibration model was built using the EEM data from all of the modified and unmodified wine vinegars in an attempt to identify a possible correlation of the matrices with the quantity of added grape-must caramel. This algorithm has the advantage of being a simultaneous model, that is, all of the components are extracted at the same time. Again, two strategies were developed: building a model with both PDOs together, and analysing each PDO separately. The NPLS accuracy for

each model is shown in Fig. 4. As indicated by the high correlation coefficient (R^2>0.921) and low RMSEC, the results of the three models were good. Moreover, the good regression results obtained by the multiway calibration agree with those obtained by other authors, due to the N-PLS algorithm having been demonstrated to be superior to unfolding methods, primarily owing to a stabilisation of the decomposition that has been demonstrated potentially to give better predictions (Bro, 1996). Moreover, another advantage is that the algorithm is fast compared with the PARAFAC approach because it consists of solving eigenvalue problems.

For regression model robustness, five of the modified samples prepared for each PDO (with intermediate concentrations of 0.20, 0.40, 0.75, 1.25 and 1.75 % of grape must caramel) were used as validation sets (included randomly in train and test) in order to test the models using known amounts of grape-must caramel. The overall prediction model accuracy obtained by the three NPLS models was very good with respect to the % of grape-must caramel predicted for these 5 samples, demonstrating the efficacy of the NPLS method. The results obtained, expressed as % of grape-must caramel, with the predicted values in brackets, as follows: 0.2(0.29), 0.4(0.47), 0.75(0.93), 1.25(1.39), and 1.75(1.85) by the global model (being these values an average of the results for both PDOs); 0.2(0.16), 0.4(0.58), 1.25(1.39), 1.75(1.74) for the *Jerez* model; and 0.2(0.18), 0.4(0.50), 0.75(0.99), 1.25(1.43) for the *Montilla-Moriles* model. The prediction results obtained for the test set are shown in Supplementary Table 2. Therefore, regarding the comparison between the measured and the predicted values obtained for these 5 samples, better results were obtained by the global NPLS model (with samples from both PDOs) than by the individual NPLS model of each PDO. This might be explained by the fact that this first model has a higher amount of samples with the same concentrations than the individual models.

In terms of the real wine vinegars, the calibration results for the RE commercial samples of both PDOs that had been shown as possibly containing more grape-must caramel or even as being less aged vinegars, again agreed with the exploratory and classification analyses performed in a previous section of this work. Thus, according to the predicted results (Supplementary Table 2), the RE samples misclassified of *Vinagre de Jerez* PDO presented amounts of grape-must caramel around 2.0%, agreeing with the predicted values of modified samples with the addition of 2.0% of grape-must caramel, whereas the rest of commercial samples had an amount of grape-must caramel lower than 1.5% and even 0.0%. Regarding the RE samples of *Vinagre de Montilla-Moriles* PDO that were classified as Modified, the predicted amount of grape-must caramel was higher than 1.0%, while the rest of the commercial samples presented a predicted value of lower than 0.5%. These values agreed totally with the observed trend of these samples in the previous PCA models. These samples were also those that showed the highest chromatographic areas for the three selected peaks.

Furthermore, in CR commercial samples that also showed a high similarity to the Modified samples with a lower amount of caramel (<0.05%), the percentages of grape-must caramel obtained by the regression models were even negative, being in agreement with this assumption (Supplementary Table 2).

All of these results confirm the ability of this multiway calibration to determine the amount of grape-must caramel in PDO wine vinegars and its ability to detect samples with an excessively high concentration. An excessive addition of grape-must caramel to a vinegar could affect its quality due to sensory changes. In fact, ranking and triangle tests, both gustatory and olfactory, were undertaken in order to assess the hypothesis of the sensory effect that adding grape-must caramel could have and in order to know the specific level of grape-must caramel that modified the sensory characteristics. Thus, the results obtained

615 by these tests showed that, in general, 0.3% was the minimum level of concentration of616 grape-must caramel at which all of the tasters perceived sensory differences in the 617 samples. However, in *Vinagre de Jerez*, grape-must caramel at a concentration of 0.05% 618 was also perceived by many testers as being different to the raw matrix. These results619 reaffirm the relevance of the present study on the importance of quantifying the grape-620 must caramel added to wine vinegars, due to the fact that changes in the organoleptic621 characteristics of wine vinegars were detected very low concentrations.

622 4 CONCLUSIONS

623 Multidimensional fluorescence coupled with a suitable chemometric method has shown624 itself to be a valuable tool for detecting and, for the first time, quantifying the addition of 625 grape-must caramel to wine vinegars without sample treatment. Thus, the methodology626 proposed provided results that were in agreement with those obtained by the conventional 627 HPLC analytical method. This, therefore, demonstrated the validity of the procedure for628 determining the amount of grape-must caramel in wine vinegars.

629 This study has also shown that the multiway regression and classification approaches630 using NPLS and NPLS-DA, respectively, provide even better results more easily and more 631 quickly than the common procedure of EEM matrices by developing PARAFAC models632 before the classification and regression models. PARAFAC has the advantage of providing 633 more information about the fluorescent compounds presented in the matrices, yet it 634 involves a more complex chemometric approach.

635 The addition of grape-must caramel is a common practice in the vinegar industry. It has636 not been studied previously because it was thought that it had no influence on the final637 vinegars. However, sensory changes in vinegars caused by adding grape-must caramel638 were also studied. The results show that low concentrations produce changes in the

organoleptic characteristics of PDO wine vinegars, reaffirming the relevance of determining the addition of grape-must caramel.

This study opens up a new means of detecting and monitoring the addition of grape-must caramel to wine vinegar, thus preventing unfair competition between wineries and brands, as well as preventing potential adulterations related to the addition of grape-must caramel. Therefore, now that the important effects that adding grape-must caramel has upon a PDO vinegar's final quality have been demonstrated, further studies are needed in order to gain greater knowledge of the subject with the aim of establishing a limit or creating a monitoring protocol regarding the addition of grape-must caramel to PDO vinegars.

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FIGURE CAPTIONS

Fig.1. Fluorescence landscapes in the form of contour plots and PARAFAC loadings (excitation/emission profiles) of each main fluorophore of different sets of samples: Calibration curves made with the Crianza "Control" wine vinegars as matrix from both PDOs (a); All the samples from both PDOs (modified and Unmodified) (b); Grape-must caramel calibration curve made with the hydroacetic matrix ((c) and (d)).

Fig.2. Score and loading plots of the principal components obtained by a PCA by using the extracted PARAFAC factors with all the Modified (MOD) and Unmodified (UNMOD) samples: for "Vinagre de Montilla-Moriles" PDO (a); for "Vinagre de Jerez" PDO (b).

Fig.3. Chromatograms corresponding to different solutions of grape-must caramel in the hydroacetic matrix showing the elution of the selected peaks (a); linear regression curves of the three compounds selected (5-HMF and two unknowns) obtained by the different percentages of grape-must caramel in hydroacetic matrix (b). HGMC= Hydroacetic matrix with the addition of grape-must caramel.

Fig.4. Figures of merit of the multiway calibration models developed with the grape-must calibration curves of both PDO considered together (a) and for each PDO individually ((b) and (c)).

SUPPLEMENTARY MATERIAL

Fig. 1. Schematic representation of the different samples and grape-must caramel curves included in the study**.**

Fig. 2. Plot of the variance explained (%), core consistency (%), number of iterations and time to carried out each model, by extracting from 1 to 6 factors, used in the selection of the best number of factors for the Vinagre de Jerez and Vinagre de

Montilla-Moriles (modified and unmodified samples) PARAFAC models, and for the PARAFAC model made with the grape-must caramel calibration curve in hydroacetic matrix.

Fig. 3. Evolution of the scores of PARAFAC factors extracted from the hydroacetic curve with the addition of grape-must caramel.

Table 1. Samples included in the study.

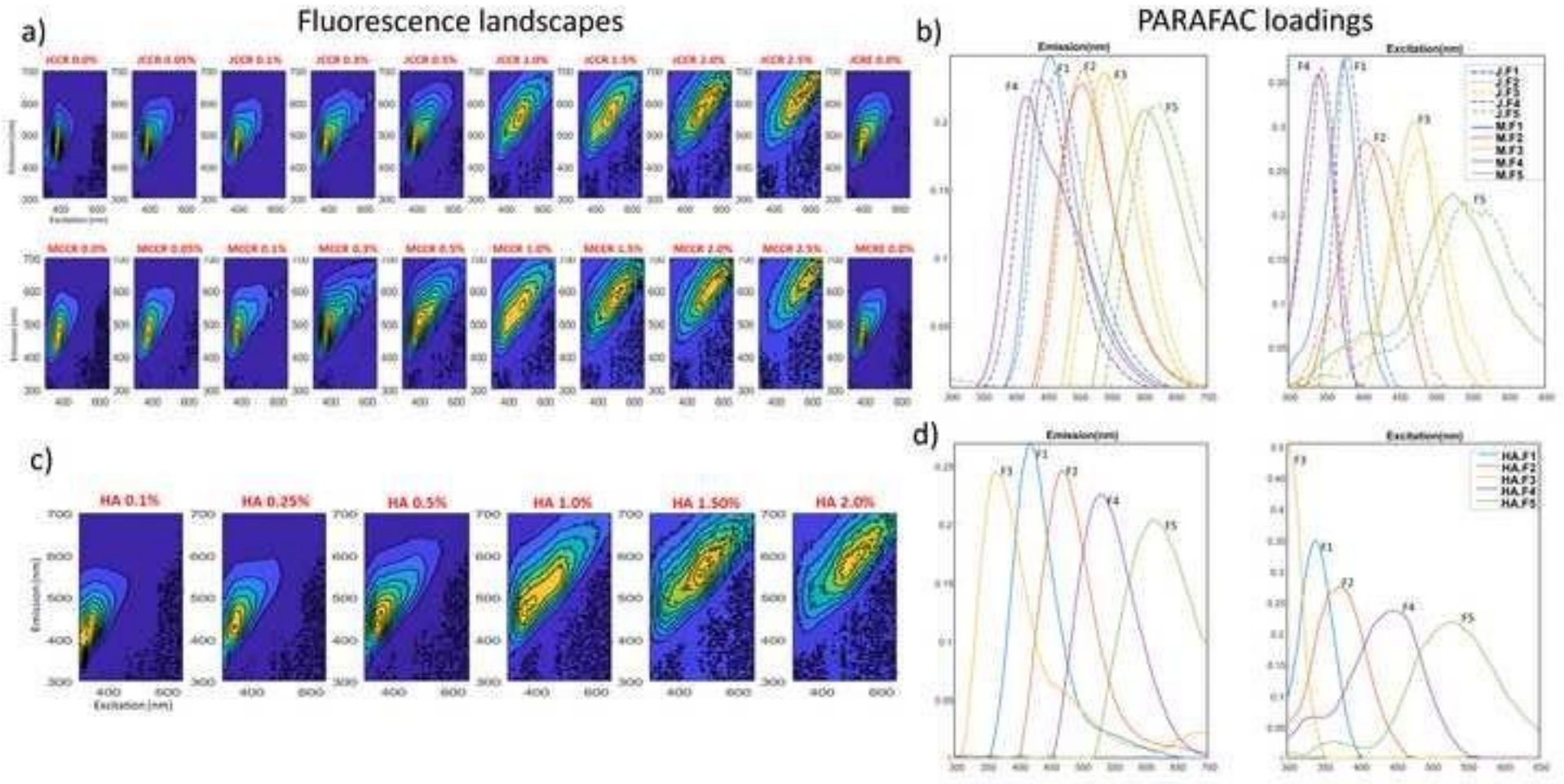
Class		Unmodified		Modified (curves made by addition of grape-must caramel)		TOTAL
		Control samples (without caramel)	**Commercial samples** (possibility of having caramel)	**Control matrix** (0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50 % v/v)	**Commercial matrix** (0.05, 0.10, 0.30, 0.50, 1.00, 1.50, 2.00, 2.50 % v/v)	
"Vinagre de Jerez"	**Crianza (JCR)**	1 (JCCR)	10 (JCR)	13	8	32
	Reserva (JRE)	1 (JCRE)	13 (JRE)	13	-	27
"Vinagre de Montilla-Moriles"	**Crianza (MCR)**	1 (MCCR)	6 (MCR)	13	8	28
	Reserva (MRE)	1 (MCRE)	5 (MRE)	13	-	19
***6% Hydroacetic matrix* (HA)**		-	-	6 (0.10, 0.25, 0.50, 1.00, 1.50, 2.00%)		6
Total		*38*		*74*		**112**

Table 2. PLS-DA and NPLS-DA classification results using the PARAFACcomponents and the EEMs, respectively.

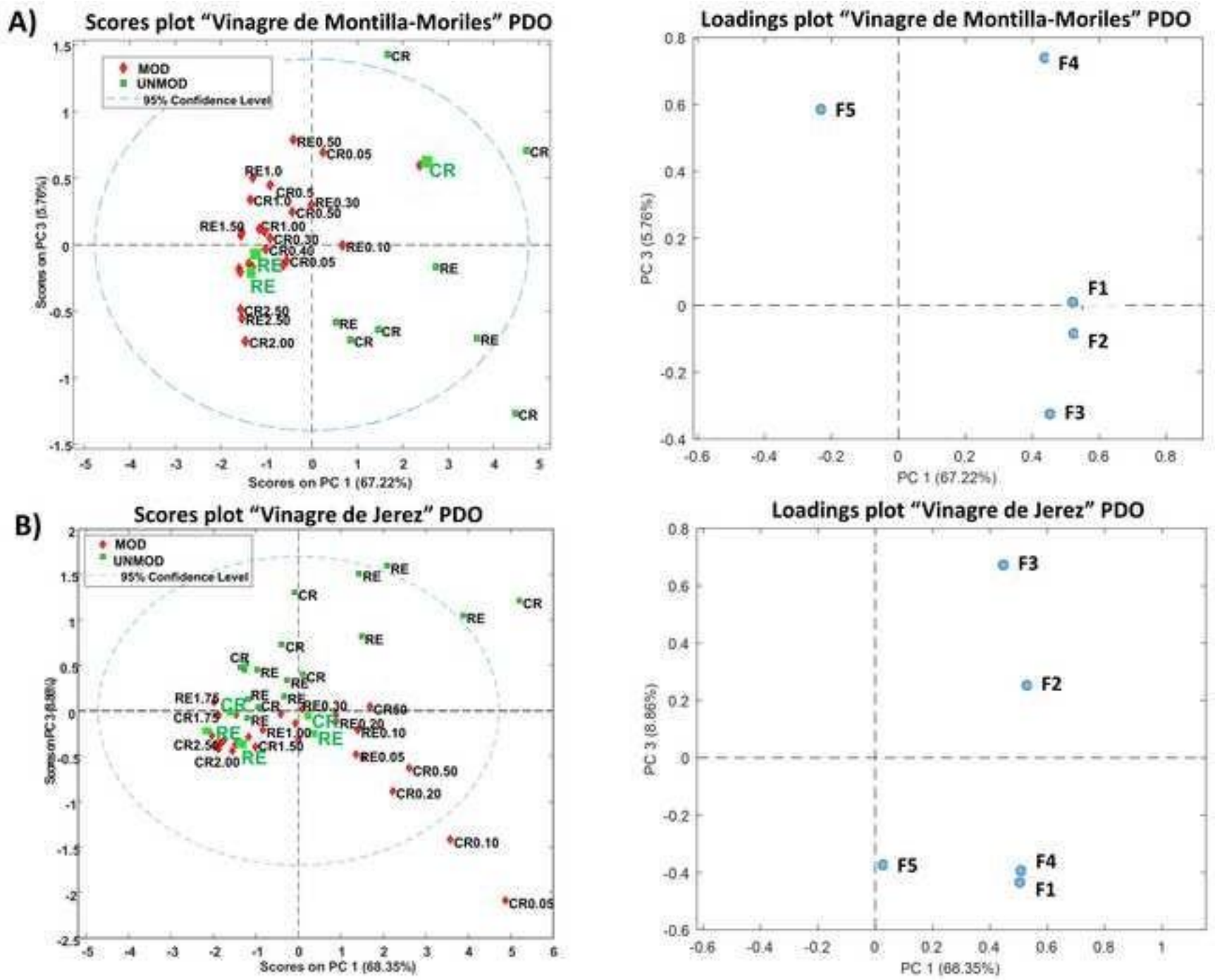
PDO	LVs		% TOTAL EXPLAINED VARIANCE		Training	% Correct Classification		SAMPLES MISSCLASIFIED	
	P	N	P	N		P	N	P	N
"Vinagre de Jerez"	4	3	99.7	99.7	Modified	100	100	0	0
					Unmodified	100	100	0	0
					Prediction	**% Correct Classification**		**SAMPLES MISSCLASIFIED**	
						P	**N**	**P**	**N**
					Modified	100	100	0	0
					Unmodified	42.86	71.43	4 (2RE,2CR)	3 (2RE,1CR)
"Vinagre de Montilla-Moriles"	**3**	**3**	96.8	96.8	**Training**	**% Correct Classification**		**SAMPLES MISSCLASIFIED**	
						P	**N**	**P**	**N**
					Modified	100	100	0	0
					Unmodified	100	100	0	0
					Prediction	**% Correct Classification**		**SAMPLES MISSCLASIFIED**	
						P	**N**	**P**	**N**
					Modified	100	100	0	0
					Unmodified	25	40.00	3 (2RE,1CR)	3 (2RE,1CR)
Both PDOs together	**5**	**3**	99.6	99.1	**Training**	**% Correct Classification**		**SAMPLES MISSCLASIFIED**	
						P	**N**	**P**	**N**
					Modified	90.70	89.47	4 (M<0.75%)	4 (M<0.75%)
					Unmodified	86.36	90.90	3 (2JRE,1JCR)	2 (1JRE,1JCR)
					Prediction	**% Correct Classification**		**SAMPLES MISSCLASIFIED**	
						P	**N**	**P**	**N**
					Modified	100	100	0	0
					Unmodified	36.36	58.33	7 (4J, 3M)	5 (3J,2M)

*Note: P= PLS-DA model; N= NPLS-DA model. LVs= Latent variables.

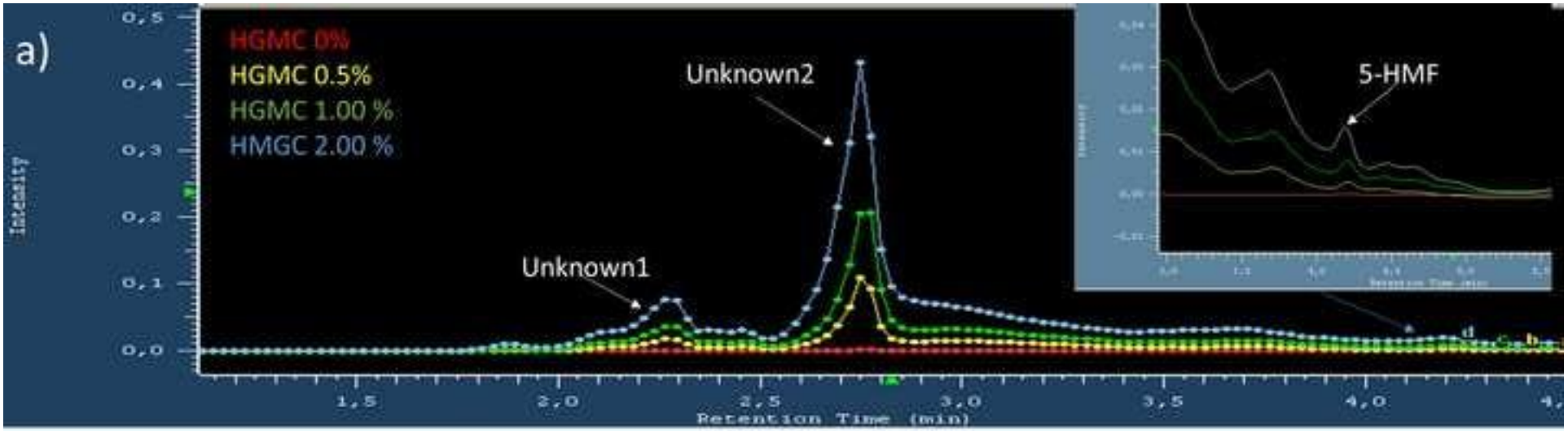
Figure(s)

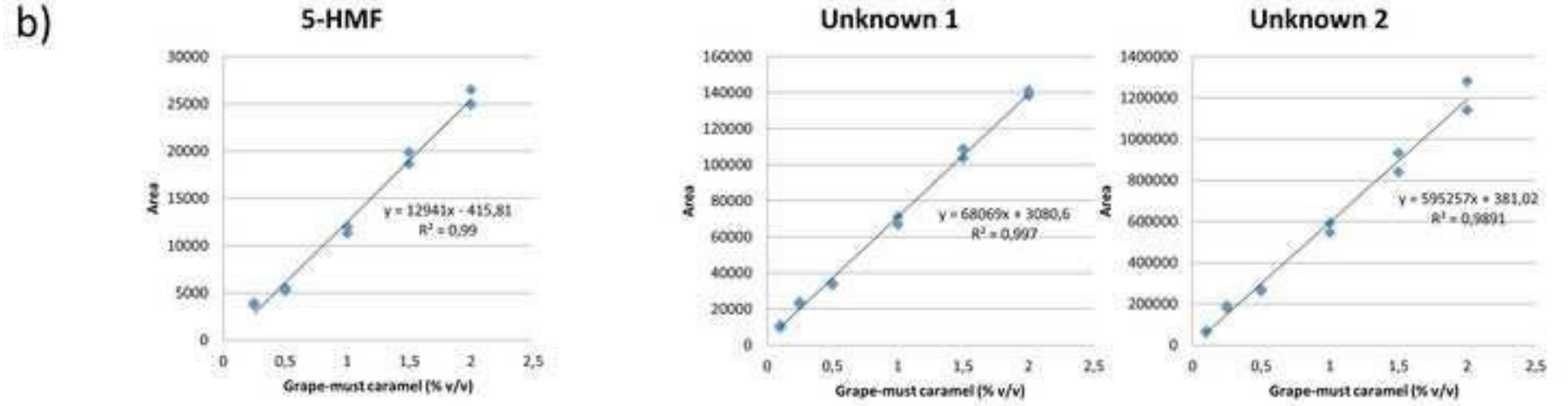

a)
Fluorescence landscapes
JCCR 0.0%
JCCR 0.05%
JCCR 0.1%
JCCR 0.3%
JCCR 0.5%
JCCR 1.0%
JCCR 1.5%
JCCR 2.0%
JCCR 2.5%
JCR0 0.0%
MCCR 0.0%
MCCR 0.05%
MCCR 0.1%
MCCR 0.3%
MCCR 0.5%
MCCR 1.0%
MCCR 1.5%
MCCR 2.0%
MCCR 2.5%
MCR0 0.0%
Emission (nm)
Excitation (nm)
b)
PARAFAC loadings
Emission(nm)
Excitation(nm)
J.F1
J.F2
J.F3
J.F4
J.F5
M.F1
M.F2
M.F3
M.F4
M.F5
c)
HA 0.1%
HA 0.25%
HA 0.5%
HA 1.0%
HA 1.50%
HA 2.0%
Emission (nm)
Excitation (nm)
d)
Emission(nm)
Excitation(nm)
HA.F1
HA.F2
HA.F3
HA.F4
HA.F5

Figure(s)

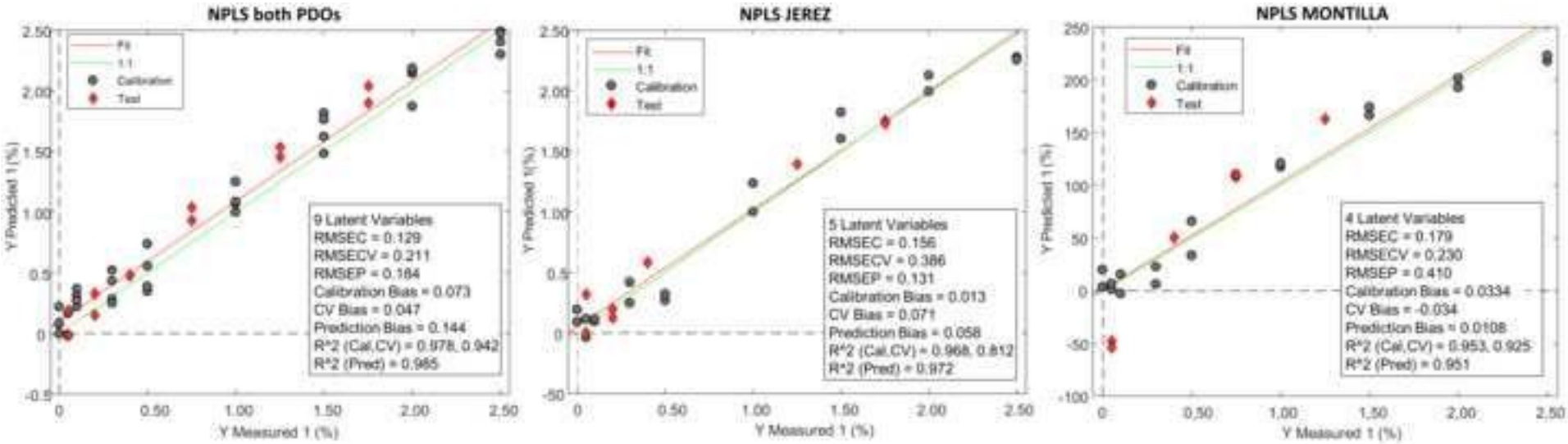

A)
Scores plot "Vinagre de Montilla-Moriles" PDO
MOD
UNMOD
95% Confidence Level
Scores on PC 3 (5.76%)
Scores on PC 1 (67.22%)
Loadings plot "Vinagre de Montilla-Moriles" PDO
F1
F2
F3
F4
F5
PC 3 (5.76%)
PC 1 (67.22%)
B)
Scores plot "Vinagre de Jerez" PDO
MOD
UNMOD
95% Confidence Level
Scores on PC 3 (8.86%)
Scores on PC 1 (68.35%)
Loadings plot "Vinagre de Jerez" PDO
F1
F2
F3
F4
F5
PC 3 (8.86%)
PC 1 (68.35%)

Figure(s)


a)
HGMC 0%
HGMC 0.5%
HGMC 1.00 %
HMGC 2.00 %
Unknown2
Unknown1
5-HMF
Intensity
Retention Time (min)


b)
5-HMF
y = 12941x - 415,81
R² = 0,99
Area
Grape-must caramel (% v/v)
Unknown 1
y = 68069x + 3080,6
R² = 0,997
Unknown 2
y = 595257x + 381,02
R² = 0,9891

Figure(s)


NPLS both PDOs
Fit
1:1
Calibration
Test
9 Latent Variables
RMSEC = 0.129
RMSECV = 0.211
RMSEP = 0.184
Calibration Bias = 0.073
CV Bias = 0.047
Prediction Bias = 0.144
R^2 (Cal,CV) = 0.978, 0.942
R^2 (Pred) = 0.965
Y Predicted 1 (%)
Y Measured 1 (%)
NPLS JEREZ
Fit
1:1
Calibration
Test
5 Latent Variables
RMSEC = 0.156
RMSECV = 0.386
RMSEP = 0.131
Calibration Bias = 0.013
CV Bias = 0.071
Prediction Bias = 0.058
R^2 (Cal,CV) = 0.968, 0.812
R^2 (Pred) = 0.972
Y Predicted 1(%)
Y Measured 1 (%)
NPLS MONTILLA
Fit
1:1
Calibration
Test
4 Latent Variables
RMSEC = 0.179
RMSECV = 0.230
RMSEP = 0.410
Calibration Bias = 0.0334
CV Bias = -0.034
Prediction Bias = 0.0108
R^2 (Cal,CV) = 0.953, 0.925
R^2 (Pred) = 0.951
Y Predicted 1 (%)
Y Measured 1 (%)

Supplementary Material

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Supplementary Material

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Supplementary Material

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Supplementary Material

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