Application of Near-Infrared (NIR) spectroscopy and chemometrics to classify and authentify wine vinegars from different Protected Designation of Origin

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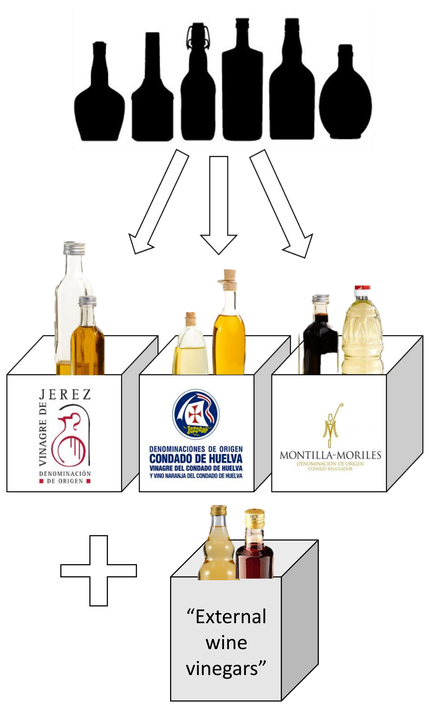
INTRODUCTION

Some wine vinegars are traditionally linked to an specific geographical area and their specifications are controlled by European regulations under a legislative system called "Protected Designation of Origin" (PDO) [1]. These vinegars have high prices in the market due to their high quality, the long aging time and the high cost of production. That explains that adulteration and unfair competition in the vinegar industry are practiced. For this reason, new analytical tools that allow rapid and inexpensive analysis are needed to protect their brands and to prevent adulteration and counterfeits. Near Infrared spectroscopy (NIR) has demonstrated to meet these characteristics. NIR spectroscopy in combination with chemometrics allows an easily treating and interpreting of the spectra, being able to perform a classification and authentication of samples without the use of chemical references [2]. For this purpose, the potential of NIR has been investigated as a rapid, inexpensive and non-destructive methodology for vinegar characterization and classification considering three of the five wine vinegar PDOs from Europe ("Vinagre de Jerez", "Vinagre de Condado de Huelva" and "Vinagre de Montilla-Moriles").

MATERIALS AND METHODS

1)Wine vinegar samples



a)

PDOs	CATEGORY	AGING TIME	CODE	Nº	Nº wineries
"Vinagre de Jerez"	Crianza	>6 months	JCR	15	34
	Reserva	>2years	JRE	15	
	Gran Reserva	>10 years	JGR	2	
	Pedro Ximenez	--	JPX	3	
	Total			35	
"Vinagre de Condado de Huelva"	Sin crianza	0 months	CSC	8	8
	Solera	>6months	CSO	9	
	Reserva	>2years	CRE	8	
	Añada	>3years	CAN	4	
	Total			29	
"Vinagre de Montilla-Moriles"	Crianza	>6 months	MCR	4	8
	Reserva	>2years	MRE	4	
	Pedro Ximenez	--	MPX	5	
	Total			13	

b)

"EXTERNAL WINE VINEGARS"	Class	Origin characteristics	Nº
"External wine vinegars" purchased from the market	"Vinagre de Jerez" PDO	"Vinagre de Jerez" PDO wineries	5
	Pedro Ximenez	Similar geographical area than "Vinagre de Montilla-Moriles" PDO	1
	Reserva and Crianza categories	Nothern Spain (Cataluña, La Rioja, Galicia)	7
	No aged	Unknown origin	3
	Total	16	

Table 1. PDO wIne vinegar samples (a) and "External" wine vinegars included in the study (b).

2) NIRs measurements

NIR spectra collected in absorption mode using an ABB Bomen IR spectrometer (Q-interline, X, Denmark), with a 1mm path length cuvette, in the range of 12000–4000 cm^{-1}, with a resolution of 8 cm^{-1} and 64 scans for both background and samples.

The spectrum of each sample was obtained in triplicate in a random sequence at room temperature (21–23 °C) by directly pipetting them into 1 mL shell vial, 40x80 mm transparent (Skandinaviska Genetec AB, Lund).

The spectrometer was interfaced to a computer with GRAMS/AI™ Spectroscopy Software (Thermo Fisher Scientific software) for spectral acquisition and exportation.

3) Statistical Analysis

- Data analysis was performed by using PLS_Toolbox 7.9.5 (Eigenvector Research Inc., Wenatchee, WA) working under MATLAB v.8.5.0 environment (The Mathworks Inc., Natick, MA).
- Smoothing (SMT) 7 point second order filtering operation, standard normal variate (SNV) method and mean centering (MC) were finally selected. Saturated and useless variables (4000-5430 cm^{-1} and 7200-6400 cm^{-1}) were removed from the whole wavenumber range of the spectra (**Fig. 1**)

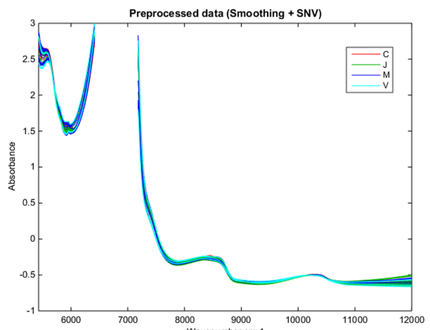


Fig1. *NIRs spectra of all PDO wine vinegars included in the study before preprocessing (smoothing and SNV)*

- Principal component analysis (PCA) was performed to study the structure of the data in an explorative manner. Then, Partial Least Squares-Discriminant Analysis (PLS-DA) was applied for wine vinegar category classification and PDO classification. Full cross validation (leave-one-out) was used as validation method and models were tested using a data set not used in the calibration model building.

BIBLIOGRAPHY

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RESULTS AND DISCUSSION

PCA models

- PCA pointed out a trend of grouping according to aging category in each PDO **(Fig. 2-A)**. Samples were placed along PC1 and PC2 from the less aged vinegars ("Crianza" >6 months aged or "Sin crianza" with 0 months) to the most aged category ("Reserva", "Gran Reserva" and "Añada"). Some overlapping was also observed due to the proximity between the ranges of aging of the categories. "Pedro Ximenez" category was perfectly separated from the rest.
- The absorption bands most involved in aging changes, and also related to sweet category, seemed to be those from 5200 to 6500 cm^{-1} **(Fig. 2-B)**.

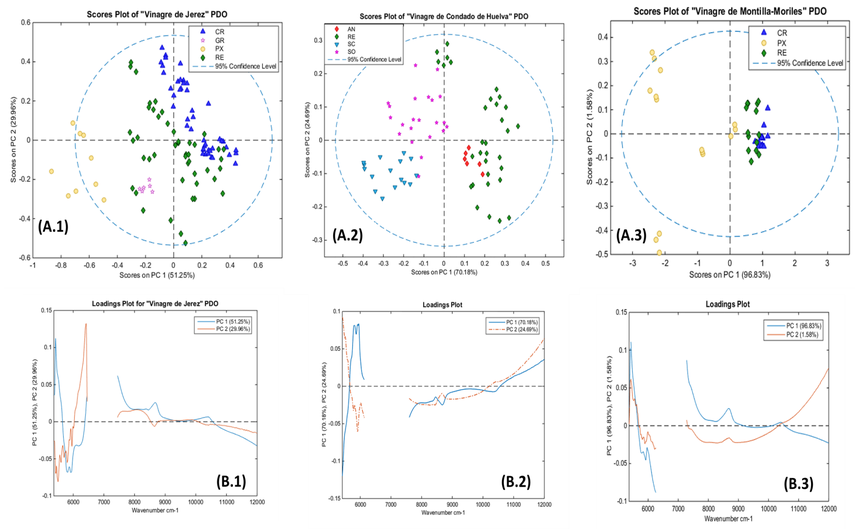

Fig.2 *PCA models of NIR spectral data of the three wine vinegar PDOs. The scores plot (A) and loadings plots (B) of the first principal components (PC1 and PC2) obtained are shown. The acronyms for the different vinegar categories are defined in Table 1.*

- To corroborate the ability of the purposed methodology in the authentication of PDO wine vinegars, some wine vinegars without a PDO indication purchased from the market, External vinegars (V), were included in the PCA models together with wine vinegars of each PDO (J, C, M) **(Fig.3).** The scores plots showed a clearly difference between PDO wine vinegars and External vinegars. Only the visual differentiation between some "Vinagre de Montilla-Moriles Pedro Ximenez" samples and one external "Pedro Ximenez" wine vinegar was not perfectly clear **(Fig.3C).**

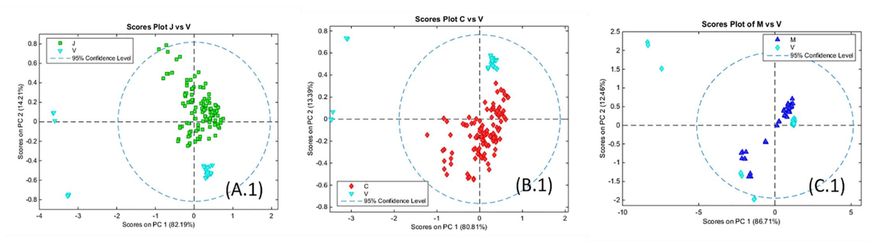

Fig.3. *Scores plots obtained by principal component analysis carried out with NIR spectra of "Vinagre de Jerez" (A.1), "Vinagre de Condado de Huelva" (B.1), "Vinagre de Montilla-Moriles" (C.1), together with wine vinegars without PDO purchased in market (named "external vinegars").*

PLS-DA models

A) CATEGORY CLASSIFICATION

The statistical parameters obtained by PLS-DA in the different models are shown in **Table 2.** The 87-100% of the samples were correctly classified. The worst classified categories were the intermediate aged ones ("Solera" for "Vinagre de Condado de Huelva" PDO and "Reserva" in the other two PDOs). These results were acceptable considering the high variability of these samples due to their wide range of aging periods reflected over their complex chemical composition.

Table 2. *Sensitivity, specificity and classification errors (%) obtained for PLS-DA classification models corresponding to the vinegar category of each Spanish PDO. The acronyms for the different vinegar categories are defined in Table 1.*

Spanish PDOs	"Vinagre de Jerez"			"Vinagre de Condado de Huelva"			"Vinagre de Montilla-Moriles"		
Nº LVs	6			2			5		
Category	JCR	JRE	JPX	CSC	CSO	CRE-CAN	MCR	MRE	MPX
Sensitivity CAL	94.4	88.6	100.0	100.0	93.3	100.0	100.0	100.0	100.0
Sensitivity CV	94.4	85.7	100.0	100.0	93.3	100.0	088.9	088.9	100.0
Sensitivity PRED	100.0	88.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Specificity CAL	97.6	88.1	98.6	97.4	82.4	100.0	100.0	095.2	100.0
Specificity CV	95.1	83.3	95.8	100.0	93.3	100.0	100.0	85.7	100.0
Specificity PRED	83.3	100.0	100.0	100.0	73.3	91.7	100.0	100.0	100.0
Class. Error CAL	3.9	11.6	0.7	1.3	12.1	0.0	0.0	2.3	0.0
Class. Error CV	5.2	15.5	2.1	1.3	12.1	0.0	5.5	12.6	0.0
Class. Error PRED	8.3	5.5	0.0	0.0	13.3	4.1	0.0	0.0	0.0

B) PDO CLASSIFICATION

After the exploratory PCA analysis, a PLS-DA was applied to confirm the ability of NIRs to authenticate and differentiate PDO wine vinegars from those without the quality indication. PLS-DA results are shown in **Table 3**. The low classification errors of prediction obtained in the models demonstrated that a good separation of PDO wine vinegar samples from the rest could be performed with the proposed methodology.

Table 3. *Sensitivity, specificity and classification errors (%) obtained for PLS-DA classification models to differentiate PDO wine vinegars from external vinegars.*

Spanish PDOs	"Vinagre de Jerez"	External vinegars	"Vinagre de Condado de Huelva"	External vinegars	"Vinagre de Montilla-Moriles"	External vinegars
Nº LVs	3		3		4	
Sensitivity CAL	100.0	100.0	100.0	100.0	100.0	100.0
Sensitivity CV	100.0	100.0	100.0	100.0	100.0	95.8
Sensitivity PRED	100.0	100.0	100.0	100.0	100.0	100.0
Specificity CAL	100.0	100.0	100.0	100.0	100.0	100.0
Specificity CV	100.0	100.0	100.0	100.0	95.8	100.0
Specificity PRED	100.0	100.0	100.0	100.0	100.0	100.0
Class. Error CAL	0.0	0.0	0.0	0.0	0.0	0.0
Class. Error CV	0.0	0.0	0.0	0.0	2.1	2.1
Class. Error PRED	0.0	0.0	0.0	0.0	0.0	0.0

CONCLUSIONS

- **The combination of NIRs and chemometrics have demonstrated that the use of the methodology proposed is a perfect combination to differentiate and authenticate wine vinegars belonging to different categories and origins.**
- **A simple exploration of the spectra by a PCA pointed out that the absorption bands most involved in aging changes, and also related to sweet category, were those from ~5200 to ~6500 cm^{-1}.**
- **PLS-DA models showed good classification results and low classification errors.**
- **In conclusion, this methodology has developed a rapid characterization, classification and authentication of the Spanish PDO wine vinegars according to the category within each PDO (aged and sweet) as well as origin (PDO wine vinegars from vinegars without this quality indication). Moreover, due to it has many advantages over other spectroscopic techniques (e.g. price, speed and portability) it could being implemented as an alternative tool for fingerprinting wine vinegar samples on a large scale.**