Classification of Wine and Alcohol Vinegar Samples Based on Near-Infrared Spectroscopy. Feasibility Study on the Detection of Adulterated Vinegar Samples

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Near-infrared (NIR) spectroscopy was used to discriminate between wine vinegar (red or white) and alcohol vinegar. One orthogonal signal correction method (OSC) was applied on a set of 73 vinegar NIR spectra from both origins and artificial blends made in the laboratory in order to remove information unrelated to a specific chemical response (tartaric acid), which was selected due to its high discriminant ability to differentiate between wine vinegar and alcohol vinegar samples. These corrected NIR spectra, as well as raw NIR spectra and 14 physicochemical variables, were used to develop separate classification models using the potential functions method as a class-modeling technique. The aforementioned models were compared to evaluate the suitability of NIR spectroscopy as a rapid method for discriminating between vinegar origins. The transformation of vinegar NIR spectra by means of an orthogonal signal correction method prompted a notable improvement in the specificity of the constructed classification models. The classification model developed was then applied to artificial vinegar blends made in the laboratory to test its capacity to recognize adulterated vinegar samples.

KEYWORDS: Wine vinegar; alcohol vinegar; multivariate classification; NIR spectroscopy; orthogonal signal correction; potential functions

INTRODUCTION

Near-infrared spectroscopy has gained wide acceptance in the field of food chemistry mainly due to its suitability for recording the spectra of solid and liquid samples at a low cost without any pretreatment and in a nondestructive way. It is also an easy-to-use, reliable, and versatile analytical method for determining different compounds in vinegar (*1, 2*) and wine or brandy samples (*3*), as well as in many other food products (*4, 5*), and for establishing certain considerations with respect to the elaboration process used (*6*). This well-known capacity has been used in this study for classification purposes.

Spanish regulations establish eight vinegar denominations: wine vinegar, cider vinegar, alcohol vinegar, cereal vinegar, malt vinegar, honey vinegar, and whey vinegar (*7*). All of them must be genuine products. Products derived from blends of final products or from the fermentation of mixtures of raw materials are illegal. The results of this study are relevant in the continuous search for safety within the vinegar industry and in the fight to curb unfair competition because they offer the possibility of differentiating different kinds of samples.

The most common fraudulent practice in the elaboration and commercialization of vinegar is the mixture of different propor-

tions of wine vinegar and alcohol vinegar. These blends are sold under the denomination of wine vinegar as if they were a pure product. Another common type of fraud is the addition of acetic acid of a nonbiological origin to different types of vinegar in order to comply with vinegar industry regulations. These adulterated products do not represent any safety risk for human health, but they constitute a fraud for consumers and are unfair practices before other vinegar producers.

The first of the adulterations mentioned above can currently be eliminated thanks to the research initiated by Vallet et al. and completed later by Remaud et al. (*8*) and Belton et al. (*9*) based on the use of site-specific natural isotope fractionation–nuclear magnetic resonance (SNIF-NMR). The SNIF-NMR technique enables the detection of an addition of 5% of synthetic acetic acid to any vinegar sample, which makes this addition not profitable for unfair producers. However, one unresolved problem already comes to the fore: the addition of acetic acid of biological origin but different from grapes. Some of the above-mentioned studies also examined these types of mixtures, mixtures of wine vinegar and alcohol vinegar, but the SNIF-NMR technique was unable to detect an addition of alcohol vinegar to wine vinegar when it was below 40–50%; this makes the fraud still possible and advantageous.

The addition of different proportions of alcohol vinegar to wine vinegar samples makes the product cheaper, and this unfair

economical advantage poses an important threat for this sector. Adulteration is difficult to detect because the addition of alcohol to the base wine prior to the commencement of the fermentation process is a common practice in the vinegar industry. The problem lies in the fact that the botanical origin of the alcohol is not always well-known. The alcohol added should come from the fermentation of skins of grapes, but sometimes its origin is fairly diverse: molasses, sugar beet, or sugar cane.

Some of the physicochemical descriptors commonly used to characterize both vinegar categories include organic acids (tartaric, L- and D-malic, lactic, acetic, citric, and succinic acids), total acids, volatile and nonvolatile acids, ash content, solids, chloride, and °Brix parameter. Some of them are included in the Spanish food safety regulations (*Reglamentación Técnico Sanitaria* − Technical Health Regulations) and were determined according to the Spanish official methods (*10*); the organic acids were determined by an HPLC method developed and validated in the laboratory (*11*). These parameters have traditionally been used to characterize and classify vinegar from different origins and processes of elaboration (*12−16*). The models computed on these parameters usually allow a good discrimination between both categories, wine vinegar and alcohol vinegar, and this fact becomes patently clear in this study.

Nevertheless, despite the good results yielded by these approaches, it is important to bear in mind that many analytical reference methods used to determine the significant physico-chemical descriptors can be fairly elaborate and/or time-consuming. It would be useful to be able to construct an accurate classification model from measurements obtained using a proven method as fast, clean, and inexpensive as, for example, near-infrared (NIR) spectroscopy has been demonstrated to be. In fact, during the past decade, one of the most common applications of NIR spectroscopy combined with pattern recognition methods has been to discriminate between samples belonging to one of several distinct groups based on spectral properties (*17−37*). Likewise, orthogonal signal correction has been used for correction of NIR spectra for classification purposes (*38*), and the potential of NIR spectroscopy for discriminating between vinegar from different origins and elaboration processes has also been investigated with relative success (*39*). Neverthe-less, although it has been proven that original NIR spectra of vinegar samples might be used directly to develop classification models with good abilities to discriminate between different origins, the observed values of interclass specificity were not as high as was hoped to ensure that extreme samples within each variety were unequivocally classified into the right category or to avoid potential errors when vinegar blends were used. In fact, the existence of optical interferences, such as light scatter in NIR data, always requires indirect multivariate calibration. Other parameters such as temperature or turbidity should also be taken into account when liquid samples are used. As a result, NIR spectra contain not only chemical but also physical information about samples and measuring conditions, which may be irrelevant and can mask the chemical information in the spectra (including information closely related to sample origin), and might deteriorate classification models developed from raw NIR spectra. Therefore, the application of a suitable preprocess-ing method, aimed at minimizing the contribution of physical effects to NIR spectra and thus enhancing the chemical information contained, could be seen as an important stage in model development and improvement.

For all of these reasons, the aim of this study is to explore the application of NIR spectroscopy for the classification and detection of adulterated vinegar samples. It offers a real

evaluation of alcohol and wine vinegar commercial samples, qualities, and diversity within each category. The potential functions method was selected as a class-modeling technique for this study, because it is a powerful method with certain specific features that enable a very comprehensive analysis of both numerical and graphical results to be performed. In this way, as an attempt to improve the classification models constructed on the basis of original vinegar NIR spectra, an orthogonal signal correction method (OSC) was applied on these raw spectra to remove information not related to a specific chemical response with a high modeling power to discriminate between vinegar origins. This specific descriptor was selected from among 14 physicochemical parameters according to their discriminant ability after the application of SIMCA. To evaluate the effect of the orthogonal corrections on sample classification, the results obtained before and after the transformation of the spectra were analyzed and compared.

MATERIALS AND METHODS

Instrumentation and Software. NIR spectra were collected for the 1100−2500 nm range using a FOSS NIRSystem 5000 liquid analyzer spectrometer (Foss NIRSystems, Silver Spring, MD) equipped with a flow cell. The instrument was controlled by a compatible PC using Vision 2.22 (Foss NIRSystems) for data acquisition.

For high-performance liquid chromatography (HPLC) measurements, a modular apparatus comprising a complete HP 1100 series system with a vacuum degasser, a quaternary pump, an autosampler, a thermostatic column compartment, and a diode array detector was used.

For regulatory analyses (*Reglamentación Técnico Sanitaria* − Technical Health Regulations), an oven (P Selecta), a furnace, a pH electrode (Crison, microH 2002, Barcelona, Spain), and a Cl⁻ ion-selective electrode (Crison, Alella, Barcelona, Spain) were used.

For °Brix measurements an Atago hand refractometer model ATC-1 with measurement range of °Brix 0−32%, accuracy of ±0.2%, and an automatic temperature compensation system from 5 to 35 °C was used.

Chemometric analysis was performed using the following software packages: Unscrambler v 8.0 (*40*), MATLAB 6.5 (*41*), and V-PARVUS package (version 2004) (*42*).

Samples. Thirty-nine vinegar samples (33 wine vinegar and 6 alcohol vinegar samples) were collected from a local vinegar industry and several supermarkets in northern Spain. All of the samples were well-labeled and claimed to be genuine products even if we can ensure only the authenticity of those samples coming from the local producer. The great difference in size of the two categories corresponds to the real differences of the different vinegar origins in the Spanish market. Thirty-four blends of wine vinegar and alcohol vinegar were prepared in the laboratory by adding 15, 30, 50, and 70% (v/v) of alcohol vinegar to nine wine vinegar samples randomly selected from the initial wine vinegar samples, covering the whole space defined by the two first principal components. Only two of the mixtures could be done from one of the wine vinegar samples due to the small amount of sample available. For this sample, the mixtures prepared were those corre-sponding to 30 and 70% of alcohol vinegar. **Table 1** shows the mean, standard deviation (SD), and maximum and minimum values of the physicochemical parameters of both sets of vinegar samples. One noteworthy feature was the wide range of some of the descriptors.

Spectroscopy Measurements. NIR spectra were recorded at 43.0 ± 0.1 °C. The samples were heated to this temperature before being introduced in the NIRSystem in order to take NIR spectra. Each spectrum is based on an average of 32 scans at 2 nm intervals within the wavelength range of 1100−2500 nm. Three replicates of each sample were taken, and the mean value was calculated. **Figure 1** shows the original NIR spectra of the samples collected. The spectral data were taken from the Vision 2.22 software and treated using Unscrambler v 8.0. Two segments of the spectrum were removed: the first, from 1880 to 2080 nm, due to the saturation of the spectrum caused by the strong combination band of O−H from water (1950 nm); and the second, from 2300 to 2500 nm, because of the low signal/noise ratio

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Table 1. Mean, Standard Deviation, and Extreme Values of the Physicochemical Parameters for Wine and Alcohol Vinegar Samples

variable	wine vinegars				alcohol vinegars			
	mean	SD	max	min	mean	SD	max	min
tartaric acid (g L^{-1})	0.675	0.281	1.576	0.265	nd^a		nd^a	nd^a
L-malic acic (g L^{-1})	0.442	0.249	1.081	0.130	0.029		0.029	nq^b
lactic acid (g L^{-1})	0.684	0.292	1.606	0.139	nq^b		nq^b	nd^a
acetic acid (g L^{-1})	59.750	0.864	62.313	57.804	60.095	0.142	60.235	59.950
citric acid (g L^{-1})	0.313	0.293	1.534	0.015	nd^a		nd^a	nd^a
succinic acid (g L^{-1})	0.569	0.333	1.619	0.180	0.054		0.054	nd^a
D-malic acid (g L^{-1})	0.635	0.789	3.292	0.021	0.012		0.012	nq^b
total acids (°)	6.087	0.088	6.304	5.923	6.118	0.076	6.182	6.033
nonvolatile acids (°)	0.091	0.036	0.183	0.033	0.006	0.002	0.008	0.003
volatile acids (°)	5.996	0.083	6.215	5.823	6.112	0.078	6.178	6.026
solids (g L^{-1})	9.786	2.168	17.407	4.535	0.871	0.311	1.220	0.625
ash (g L^{-1})	1.772	0.429	3.067	0.900	0.399	0.239	0.673	0.230
chloride (g L^{-1} NaCl)	0.257	0.180	0.839	0.051	0.040	0.036	0.081	0.016
°Brix (%)	4.19	0.23	5.00	3.80	3.37	0.06	3.40	3.30

^{a} nd, not detectable, values under the detection limit. ^{b} nq, not quantifiable, values under the quantification limit.
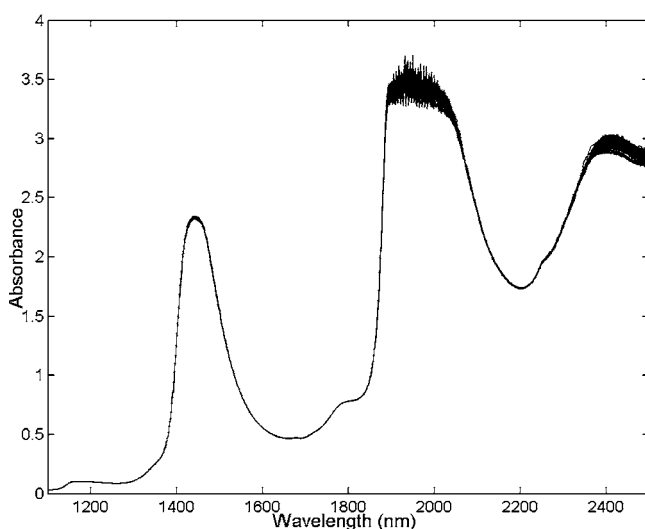
Figure 1. Raw NIR spectra of the wine and alcohol vinegar samples.

value. The mean values were exported for further mathematical preprocessing and class-modeling step.

Reference Analysis. Regulatory analyses (total acids, volatile and nonvolatile acids, solids and ash contents) were analyzed in accordance with the *Official Methods of Analysis* for vinegars (*10*).

Chloride was determined by direct potentiometric determination with a Cl$^-$ ion-selective electrode as a practical application of the Nernst equation. It is based on the use of small quantities of both sample and standard and the generation of a standard curve by serial addition.

The Brix (%) parameter shows the concentration percentage of the soluble solids content in a sample (water solution). Soluble solids content is the total of all the solids dissolved in the water, including sugar, salts, protein, acids, etc., and the measurement reading is the total sum of these. Basically, Brix (%) is calibrated to the number of grams of cane sugar contained in 100 g of cane sugar solution. Hence, when a vinegar sample is measured, Brix (%) represents the "sugar equivalents" in the vinegar sample.

Organic acids were determined by HPLC with a DAD detector. All of the standard solutions and vinegar samples were passed through a 0.7 μm glass microfiber GF/F supplied by Whatman (Whatman International Ltd., Maidstone, U.K.) prior to injection into the HPLC system. The column used was a Zorbax SB-C18 with a stable bond packaging suitable for working at low pH values. The mobile phase was 0.009 M potassium dihydrogen phosphate (adjusted to pH 2.06 with phosphoric acid)/methanol (92:8 v/v) at a flow rate of 0.64 mL min^{-1} at 25 °C and a working pressure of 90 bar (1 bar = 10^5 Pa). Detection was performed by measuring UV absorption at 210 nm. This chromatographic method was developed and optimized in the laboratory

using experimental design and desirability functions as a multicriteria-decision-making method for the separation, determination, and quantification of these compounds (*11*).

Chemometrics and Data Analysis. The whole data matrix had 73 objects, 498 spectral variables (NIR absorbance values), and 14 physicochemical variables. The initial set of 73 objects was divided into two sets: the training set used to construct the classification models and the external test set used to test the models and express the prediction capacity of the computed models. The objects assigned to the external test set were randomly selected covering the experimental domain from the initial set of 73 samples and accounted for 13.7% of the total number of samples.

The pretreatment method applied to the data was orthogonal signal correction (OSC). OSC was applied to avoid all of the effects and interferences in the response that presented zero correlation with the reference value **y** (*43*−*45*). Since the introduction of OSC, a number of different approaches that have attempted to improve or modify this have been presented in the literature (*46*−*48*). In the present study, the original method proposed by Wold (*43*) and implemented in the *osccal* function of the PLS Matlab toolbox was used (*49*).

Two class-modeling techniques were used in the study: SIMCA and the potential functions method. SIMCA was the first class-modeling technique introduced in chemistry by Svante Wold (*50*). SIMCA does not make any hypothesis on the distribution of variables; each category model is developed independently, and no information in the external categories is used. The mathematical model of the category is based on the principal components of the category; these are normally obtained as eigenvectors of the correlation coefficient matrix of the category, that is, by using the data after separate category autoscaling or column centering as in our case.

Potential functions methods are classification and modeling methods when there is no normal data distribution. Rather than estimating the probability density function using parameters such as mean and standard deviation, these methods use the local density of the objects in the training set; these objects are then used to compute the probability density. The probability function is computed as the sum of individual contributions of the objects in the training set. Consequently, these methods are known as potential functions techniques (*51*). The individual contribution can have different shapes, a Gaussian function being one of the most commonly used. Each Gaussian function contains a smoothing parameter *u* that determines how broad the individual contribution is. The probability distribution function in a point **x** is the sum of the individual contributions, and the selection of the smoothing parameter is performed by a cross-optimization procedure (e.g., leave-one-out) (*52*). In the case of multivariate data, the individual potential in one point **x** must take into account the **V** variables. The smoothing parameter is substituted by the product of the smoothing factor *k* for the standard deviation of each variable, so only the parameter *k* is optimized. When several categories are studied, as in the present case, the smoothing factor is computed for each category (*52*).

Since the appearance of potential functions as a classification method in analytical chemistry (1950s), several classification methods and clustering procedures have been developed based on that method (*53, 54*), also being applied for more than simply classification purposes, such as selecting representative subsets of samples (*52*).

Usually, the class-modeling technique based on potential functions applied in this study validates the predictive ability of classification models constructed by cross-validation, because when one is working with potential functions the waste of objects to make up an external evaluation set is not recommended. However, it is known that orthogonal signal correction methods can produce a notable overfitting when applied on the spectra forming the training set. For this reason, although all potential functions classification models were constructed by cross-validation, we decided to also validate the actual predictive abilities of resulting models by testing their performance on an external test set, simply to control and avoid a possible overfitting that could inherently appear due to the orthogonal signal correction method applied. Hence, the results are expressed as correct classification results in calibration, validation by cross-validation, and validation by external test set. The complexity of the models in all cases was chosen according to the results obtained in the cross-validation process. The models performed were then tested on the external test set.

The quality of the results provided by the different class models constructed was compared according to the following evaluation parameters:

Category classification/prediction rate

$$R_C = \frac{m_{cc}}{N_C} \times 100 \tag{1}$$

This equation was applied both in classification and in prediction, where m_{cc} is the correct classification number and N the total classification or prediction number that corresponds to the total number of objects in a category C (during cross-validation an object is classified many times).

McNemar's test was used to evaluate the significance of the results obtained by the different class models. When two models, A and B, were compared, McNemar's value is calculated as

$$\text{McNemar's value} = \frac{(|n_{01} - n_{10}| - 1)^2}{n_{01} + n_{10}} \tag{2}$$

where n_{01} is the number of samples misclassified only by algorithm A and n_{10} is the number of samples misclassified only by algorithm B. McNemars's test (*55*) is a particular case of Fisher's test (*56*). The aim is to have a statistical procedure to decide if two methods have the same accuracy. Two algorithms, A and B, are trained and validated with the same sets. The null hypothesis is that the two algorithms A and B have the same error rate, which means that they have the same percentage of well-predicted samples (*57, 58*). McNemar's test is based on a χ^2 test with one degree of freedom (if the number of samples is >20). The critical value with a 5% level of significance ($\chi_{(1,0.95)}^2$) is 3.84. If McNemar's value is greater than this value, the null hypothesis is false and the two algorithms are significantly different. In the present work, McNemar's test was applied to both calibration and test sets jointly due to the reduced number of samples in the test set. In the final part of the potential functions program all of the models are tested on the whole data set and given a value of "accepted" or "rejected" for each of the class models computed. This information was used to perform McNemar's test.

Graphical tools, such as isopotential lines and Coomans plots, were also used to analyze the goodness of the models.

RESULTS AND DISCUSSION

Original NIR Spectra. In the first part of the study only the two "pure" categories (wine and alcohol vinegar) were considered, accounting for 39 samples that have been divided at random into training and test sets.

Table 2. Calibration and Prediction Classification Rates for the Potential Functions Models Computed on the Diverse Variables (Physicochemical or NIR) for Wine and Alcohol Vinegar Categories[a]

	1 (wine vinegar)			2 (alcohol vinegar)		
category	com- puted	CV[b]	external test set	com- puted	CV[b]	external test set
raw NIR variables	92.3	92.31	85.7	40.0	60.0	0.0
OSC1(tartaric)	100.0	100.0	85.7	100.0	100.0	100.0
OSC2(tartaric)	100.0	100.0	85.7	100.0	100.0	100.0
OSC3(tartaric)	100.0	100.0	71.4	100.0	100.0	100.0
14 physicochemical variables	100.0	100.0	100.0	100.0	100.0	100.0
tartaric acid	100.0	100.0	100.0	100.0	80.0	100.0

[a] Results expressed as percentage of correct classification. [b] CV, results in cross-validation.

Table 3. Sensitivity and Specificity Values for the Potential Functions Models Computed for Categories 1 (Wine Vinegar) and 2 (Alcohol Vinegar)[a]

	1 (wine vinegar)		2 (alcohol vinegar)	
category	sensitivity	specificity for class 2	sensitivity	specificity for class 1
raw NIR variables	92.3	0.0	100.0	61.5
OSC1(tartaric)	96.2	40.0	100.0	100.0
OSC2(tartaric)	92.3	100.0	100.0	100.0
OSC3(tartaric)	92.3	100.0	100.0	100.0
14 physicochemical variables	96.2	100.0	100.0	100.0
tartaric acid	96.2	100.0	80.0	100.0

[a] Results expressed as percentage: for sensitivity, the percentage of objects correctly identified by the mathematical model; for specificity, the proportion of objects not belonging to a category and classified as foreign.

Table 2 summarizes the classification and prediction percentages corresponding to the class models developed on the basis of mean-centered original NIR spectra of vinegar samples using the potential functions method with two principal components (PCs) for the two-class problem analyzed. Two PCs were used to compute the model to reach the maximum correct classification rates in the cross-validation process, accounting for the 73% of the explained variance of the system. The results can also be expressed in terms of sensitivity and specificity. Sensitivity is the proportion of objects belonging to a category that are correctly identified by the mathematical model; specificity is the proportion of objects foreign to the category that are classified as foreign. **Table 3** shows the sensitivity and specificity for the two categories studied. The specificity of the model of category 1 for category 2 is 0.0%, which means that none of the objects of the second category are classified as foreign. These two parameters (sensitivity and specificity) are also significant because it is important not only that one model accepts samples belonging to the category but also that the model rejects samples belonging to other categories.

These numerical results can be also confirmed graphically. **Figure 2a** shows the Coomans plot corresponding to the classification model constructed from raw NIR spectra with complexities of two PCs. Bearing in mind the categories studied here, the bottom right zone corresponds to the samples accepted by the alcohol vinegar class model, whereas the upper left zone corresponds to wine vinegar samples. Wine vinegar samples are represented by a circle and alcohol vinegar samples by a triangle, and the objects in the external test set are plotted as a cross. The large number of samples displayed in the uncertainty
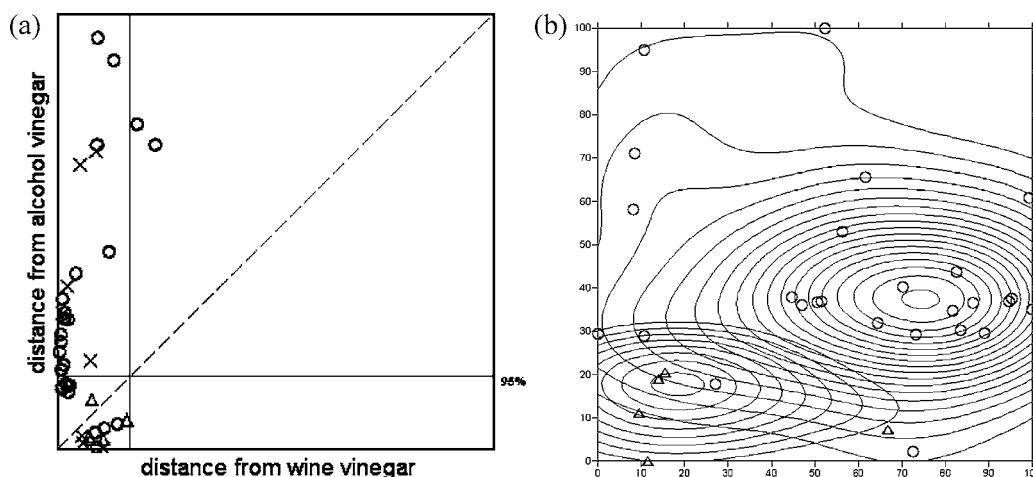
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Figure 2. (**a**) Coomans plot and (**b**) isopotential lines plot of the class-models constructed over the mean-centered raw NIR data: (○) wine vinegar; (△) alcohol vinegar; (×) test set.

area belonging to both models demonstrates the low degree of specificity of these models; all of the samples belonging to the alcohol vinegar category are in this area. The potential functions method also enabled us to obtain potentials for contour plots (isolines), taking into account all the objects and categories, which was very useful as a visualizing method. **Figure 2b** shows the isopotential lines plot corresponding to the potential functions model of both wine vinegar (circles) and alcohol vinegar (triangles) categories. As can be seen, class models relating to both vinegar categories did not appear perfectly separate, showing a high degree of overlapping.

In view of the numerical and graphical results provided by the optimal classification model developed from raw NIR spectra, several remarks should be made. The discrimination ability exhibited by the selected model cannot be considered to be satisfactory for classifying vinegar from two different origins—wine and alcohol. The relatively short distance between the class models (exhibited in the Coomans plot and confirmed by the clear overlapping between isopotential lines) could reveal some potential problems for classification of extreme samples within each category or blends of varieties in the future. This finding underscores the importance of the objectives pursued by the present study, that is, trying to improve the final classification model in terms of both specificity and sensitivity to enable a more accurate practical application.

Chemical Variables: Selection of a Discriminant Descriptor. After the relative specificity problems exhibited by the previous models constructed on the basis of the raw NIR spectra, new studies were performed on 14 physicochemical variables to determine the potential ability for discriminating between the categories. These variables included the common parameters included in the Technical Health Regulations and the organic acids and °Brix parameter related to the carbohydrate content. As a first attempt, SIMCA was performed over the two vinegar categories (wine vinegar and alcohol vinegar) and the 14 physicochemical variables, the main objective being to determine the modeling power and the discriminating power of each variable. The variables total acids and nonvolatile and volatile acids achieved the higher modeling power for category 1 (wine vinegar), whereas solids and volatile acids were the variables with higher modeling values for category 2 (alcohol vinegar). With reference to discriminating power, tartaric acid proved to be the most discriminatory variable, followed by citric acid. The other 12 variables displayed less discriminating power between the categories.

The fact that tartaric acid is the most discriminating descriptor was reasonable. Tartaric acid originally comes from grapes, and this is the main reason this variable discriminates between these two vinegar categories. A number of hypotheses can be proposed with regard to tartaric acid content in wine vinegar samples. Tartaric acid ranged from 1.576 to 0.265 g L^{-1} in the commercial wine vinegar studied. Normally, the tartaric acid content in wines of 11−14 alcoholic grades is variable, ranging from 3 to 1.2 g L^{-1} (*59, 60*). Considering that the normal base wine used in the vinegar industry has an average of nine alcoholic grades and considering all of the dilution processes during vinegar elaboration, a normal expected range for tartaric acid in wine vinegar would be 0.45−1.15 g L^{-1}. Low values of tartaric acid content are indicative of an extra dilution process or any adulteration process.

Again, the potential functions method was used to compute the classification models for both vinegar categories on the basis of these 14 physicochemical descriptors, and the classification results in calibration and prediction are shown in **Table 2**. **Table 3** shows the sensitivity and specificity results. From the results in the tables it can be observed that the models constructed on the physicochemical variables are able to neatly separate both categories. The same good results are obtained when the class models are computed over the most discriminant variable, tartaric acid (see **Tables 2** and **3**).These results confirm the suitability of the tartaric acid as discriminant variable.

Corrected NIR Spectra. OSC was applied to the original NIR data, removing one, two, and three orthogonal factors (latent variables, PCs, or components in general) unrelated to the tartaric acid content. The corrected spectra were then used to construct the respective potential functions class models. The results, expressed as correct classification and prediction percentages, are shown in **Table 2**. The complexities of the models were two in all cases and accounted for 50.6, 63.5, and 66.2% of explained variance, respectively, when one, two, or three orthogonal components have been removed.

Considering the spectra corrected by OSC, it can be seen that, after removal of two orthogonal components, the results in terms of classification and prediction, as well as selectivity and specificity, were excellent. The removal of three orthogonal components generated a slight overfitting because the percentage of correct classification in prediction for the test set for category 1 was slightly lower than the results obtained for two orthogonal components. These numerical results were visually confirmed by Coomans and isopotential lines plots. **Figure 3** shows the
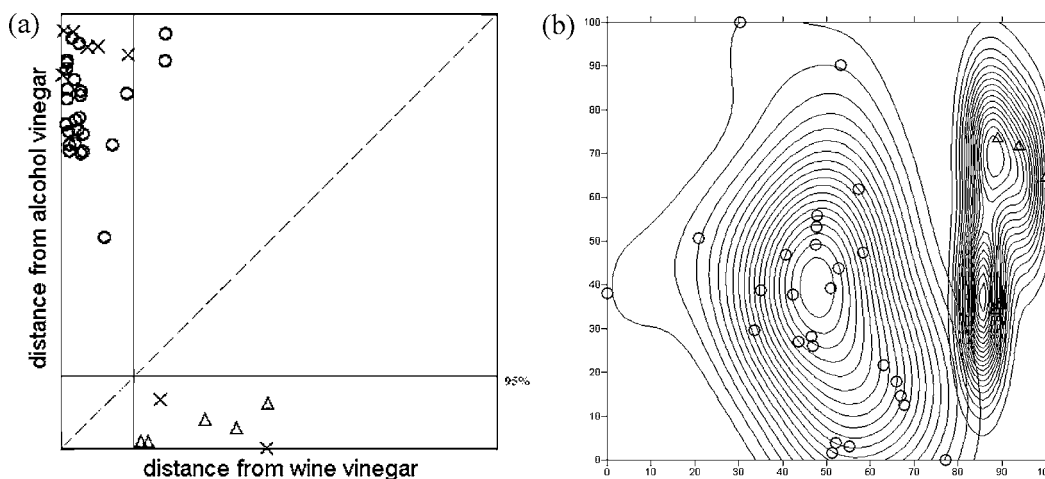
Figure 3. (**a**) Coomans plot and (**b**) isopotential lines plot of the class-models constructed over the OSC-corrected NIR spectra after elimination of two orthogonal components: (○) wine vinegar; (△) alcohol vinegar; (×) test set.
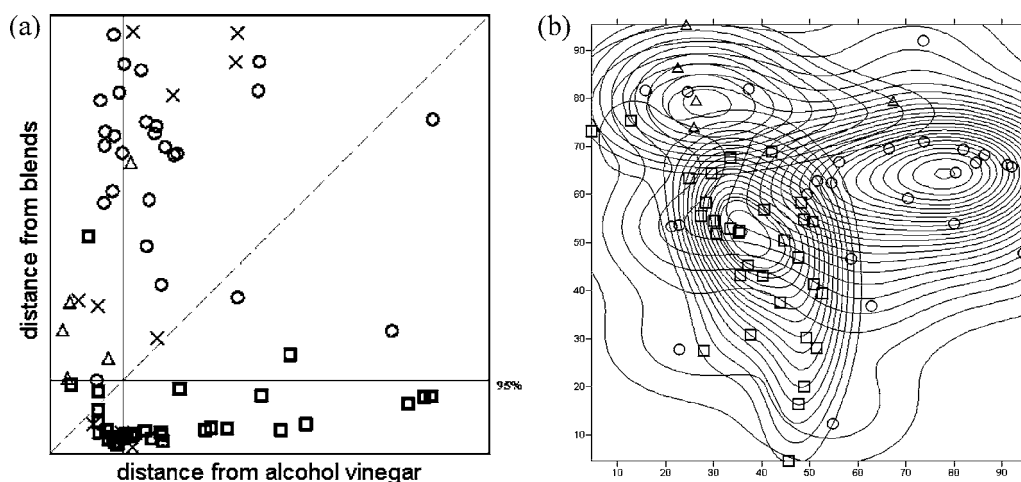

Figure 4. (**a**) Coomans plot and (**b**) isopotential lines plot of the class-models constructed over the mean-centered raw NIR spectra of wine vinegar, alcohol vinegar, and blends: (○) wine vinegar; (△) alcohol vinegar; (□) blends; (×) test set.

Table 4. Comparison of the Different Potential Functions Methods with McNemar's Test[a]

McNemar's value	raw NIR variables	OSC1-(tartaric)	OSC2-(tartaric)	OSC3-(tartaric)	14 variables	tartaric acid
raw NIR variables		**13.07**	**15.06**	**12.50**	**15.42**	**16.06**
OSC1(tartaric)			0.25	0	1.50	1.33
OSC2(tartaric)				0	0.25	0
OSC3(tartaric)					0.80	0.25
14 variables						0
tartaric acid						

[a] Two categories were studied, wine and alcohol vinegar. Boldface numbers indicate that the two methods are significantly different.

Coomans plot and the isopotential lines plot for the model computed on the OSC-corrected NIR variables once two orthogonal components have been eliminated. **Table 3** shows a high degree of interclass specificity, sensitivity, and a patently clear separation between classes, similar to that obtained when using tartaric acid content as a discriminating descriptor and considerably improved with regard to the model constructed from raw spectra.

The significance of the differences can be statistically studied by McNemar's test. In **Table 4** are given the McNemar's values for the different approaches performed. Note that all of the methods computed on the OSC-corrected variables and the physicochemical variables are significantly different from the

method computed on the raw NIR spectra. However, the OSC-corrected methods with one, two, or three components and the methods based on the physicochemical variables are not significantly different.

Blends. At this point, the several artificial blends of wine and alcohol vinegars with 15−70% adulteration in percentage (v/v) of alcohol vinegar prepared in the laboratory are added to the data of the two "pure" categories. The potential function models were computed on a system of three categories. The results are presented according to the calibration and prediction rates for the three categories: wine vinegar, alcohol vinegar, and blends. Panels **a** and **b**, respectively, of **Figure 4** show the Coomans plot and the isopotential lines plot including the blends of both vinegar origins. The axes of the Coomans plot represent the categories of alcohol vinegar (triangles) and blends (squares) so that the upper right quadrant corresponds to the samples rejected by the two models represented. Thus, in this case, the samples that should be in this quadrant are the genuine wine vinegar samples (circles). As in the previous case, the samples included in the external test set are represented by a cross. The isopotential lines plot shows that a strong overlapping between the three categories was obtained. In this case five components are needed to get the best classification rate explaining 88.8% of the variance of the system.

Better results were obtained after the OSC correction taking into account the tartaric acid content in the samples had been

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Table 5. Calibration and Prediction Classification Rates for the Potential Functions Models Computed over Three Categories: Wine Vinegar (1), Alcohol Vinegar (2), and Blends of the Previous Two (3)[a]

category	1 (wine vinegar)			2 (alcohol vinegar)			3 (blends)		
	computed	CV[b]	external test set	computed	CV[b]	external test set	computed	CV[b]	external test set
NIR variables	100.0	96.2	100.0	80.0	60.0	0.0	93.8	93.8	100.0
OSC1(tartaric)	100.0	96.2	85.7	100.0	80.0	0.0	96.9	93.8	100.0
OSC2(tartaric)	96.2	100.0	85.7	100.0	60.0	0.0	96.9	96.9	100.0
OSC3(tartaric)	92.3	92.3	85.7	80.0	60.0	0.0	96.9	96.9	100.0

[a] Results expressed as percentage of correct classification. [b] CV, results in cross-validation.
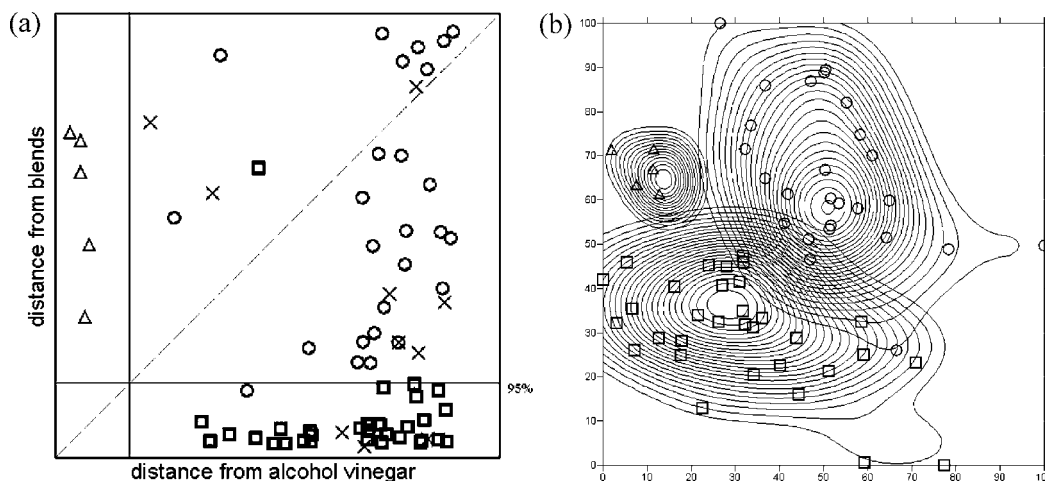
Figure 5. (**a**) Coomans plot and (**b**) isopotential lines plot of the class-models constructed over the OSC-corrected NIR spectra (two OSC components) of wine vinegar, alcohol vinegar, and blends: (○) wine vinegar; (△) alcohol vinegar; (□) blends; (×) test set.

performed. The potential functions model was computed by considering three categories: wine vinegar, alcohol vinegar, and blends. The data were column-centered separately by category. **Table 5** shows the classification rates obtained after the potential functions method had been applied to the three categories studied. The sensitivities and specificities results were also considered to choose the method obtained by the removal of two orthogonal components as the best classification strategy. Five components accounting for 86.4% of the explained variance were needed to obtain good classification results. The Coomans plot in **Figure 5a** shows a better separation between categories 2 (alcohol vinegar) and 3 (blends), but some blend samples rejected by the represented model and mixed with the wine vinegar samples can still be seen. This can be verified in **Figure 5b**, where the alcohol samples can be well-distinguished from the other samples, although wine vinegar samples and blends still overlap despite the fact that two clusters can be distinguished, one for wine vinegar and another for blends. The reason for this impossibility of neatly distinguishing between commercial wine vinegar and blends of wine and alcohol vinegar is the wide range of concentration presented by the descriptor tartaric acid in both groups of samples. Extreme samples of both categories, wine vinegar and blends, are mixed up. This is significant of the quality and genuineness of the commercial wine vinegar samples and opens up a new area of research in the study and quantification of vinegar adulteration. Besides, it should be stated that the blends made in the laboratory contain some of the same chemical variability as the samples used to compute the class models. This fact added to the hypothesis of considering the commercial samples genuine, makes of this work a feasibility study and not an absolute methodology to determine fraudulent vinegar samples. Further studies are being carried out on this.

Table 6. Comparison of the Different Potential Functions Methods with McNemar's Test

McNemar's value	raw NIR variables	OSC1-(tartaric)	OSC2-(tartaric)	OSC3-(tartaric)
raw NIR variables		**4.32**	**15.61**	1.64
OSC1(tartaric)			**5.88**	0.19
OSC2(tartaric)				**7.68**
OSC3(tartaric)				

[a] Three categories were studied: wine vinegar, alcohol vinegar, and blends. Boldface numbers indicate that the two methods are significantly different.

Again, in **Table 6** are presented McNemar's values computed on the whole data set. In this case, the differences between the models performed on OSC-corrected NIR variables eliminating one or two orthogonal components are significantly different from the models performed on the original NIR variables. However, OSC correction removing three orthogonal components results in a model not significantly different from the original one. The differences between OSC1(tartaric) and OSC2-(tartaric) approaches are significant, as are the differences between OSC2(tartaric) and OSC3(tartaric). Note that the McNemar's value when the original method (raw NIR variables) and the OSC1(tartaric) method are compared is close to the critical value of 3.84; the comparison between OSC1(tartaric) and OSC3(tartaric) is not significant, so it can be concluded that the OSC2(tartaric) method yields the best results, being significantly different from the original model.

Conclusions. This study has shown that the transformation of vinegar NIR spectra using an orthogonal signal correction method, taking into account in this correction a chemical response closely related to sample origin, prompted a substantial improvement in the quality of the constructed classification

models in comparison with the model developed on the basis of original spectra. The correction of NIR spectra based on the tartaric acid content provides better discrimination between wine vinegar and alcohol vinegar and good results in terms of both classification/prediction ability and stability.

On the other hand, it is important to clarify that the strategy introduced would not imply any additional effort in relation to analytical determinations in future samples to be characterized. The measurement of tartaric acid content values was required only for samples forming part of the calibration set. Once calibration NIR spectra have been corrected by applying the orthogonal signal correction method, the same correction can be directly applied to future sample spectra without the need to measure any reference tartaric acid content value. In this way, from a practical application standpoint, the classification methodology proposed would rely only on NIR measurements. Therefore, in view of the results obtained, it could be stated that the advantages offered by the improved classification models in terms of both specificity and reliability compensate for the need to measure an extra chemical property (tartaric acid) in the case of the calibration samples.

When the method developed was applied to blends of wine and alcohol vinegars, three clusters were observed. These results can be indicative of the quality of commercial wine vinegar samples used in the calibration set to construct the class models. This study presents a feasibility study for the identification of adulterated vinegar samples.

The promising results obtained in this study mean that a similar procedure could be considered in future applications to quantify different blends of varieties to identify and determine the percentage of adulteration in fraudulent mixtures.

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