Chemical characterization of wine vinegars belonging to the *Vinagre de Montilla-Moriles* protected designation of origin, using near infrared spectroscopy

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Abstract

Spanish wine vinegars belonging to *Vinagre de Montilla-Moriles* protected designation of origin (PDO) must satisfy some chemical characteristics. These characteristics are mainly responsible of their high and exceptional quality. This study assessed the potential of near infrared spectroscopy (NIRS) as a non-destructive technology for characterizing wine vinegars belonging to this PDO. A total of 107 samples were used to predict major chemical quality parameters (volumic mass, reducing sugars, total acidity and pH) using a scanning monochromator (spectral range 400-2500 nm) with the spinning module, working in transflectance mode. The models developed showed values for the coefficient of regression for cross-validation between 0.95 and 0.99 for volumic mass, reducing sugars and total acidity. Therefore, the results confirm that NIRS technology combined with linear regression strategies such as the modified partial least squares (MPLS) regression can indeed respond to the needs of the vinegars cellars and help them to measure the commonest chemical quality parameters of wine vinegars belonging to *Vinagre de Montilla-Moriles* (PDO), especially in the case of vinegars with different sugar contents (dry, semi-sweet, sweet and balsamic). However, the number of samples as well as their variability should be increased in order to obtain more robust models.

Keywords: *Vinagre de Montilla-Moriles* (PDO), NIR spectroscopy, volumic mass, reducing sugars, total acidity.

Introduction

Wine vinegar is a product of the gastronomic culture of Mediterranean wine-producing countries. Its versatility as a preservative, acidifier or condiment has meant that it is still widely used to this day, and it has recently assumed greater importance due to the health benefits associated with its consumption (Tesfaye et al. 2002; Paneque et al. 2017).

The wide variety of vinegars existing in the market and the rise in their demand call for a more thorough definition of its main chemical and sensory parameters, in order to set up a rigorous system of quality control to regulate the quality of the vinegar, the acetification system used and, in the final step, the wooden barrels used for the aging process (Morales et al. 2001; De la Haba et al. 2014).

Since the quality of a vinegar is closely linked to its commercial value, it is necessary to evaluate its main chemical and sensory characteristics in order to guarantee high levels of quality. Objective quality standards enable vinegar producers to attain high levels of quality, which, in turn, will increase the profits of the vinegar industry.

In the European Union, several high quality vinegars, generally linked to particular geographical areas, are protected by a legal framework known as a Protected Designation of Origin (PDO) (OJEU 2006). Through this scheme, in January 2015, the wine vinegar *Vinagre de Montilla-Moriles* was registered in the European Union Register of protected designations of origin and protected geographical indications (OJEU 2015). This Regulation defines *Vinagre de Montilla-Moriles* as either wine vinegar obtained by the acetous fermentation of certified 'Montilla-Moriles' PDO wine or, where appropriate, vinegar from wine vinegar obtained by the acetous fermentation of certified 'Montilla-Moriles' PDO wine, with the addition of certified must of that wine, followed by ageing.

In addition, in the Annex to the Commission Implementing Regulation, the protected products and their characteristics of vinegars belonging to *Vinagre de Montilla-Moriles* (PDO) were set down (OJEU 2015). This document establishes the chemical and sensory quality of the vinegars, guarantees the quality for consumers and protects them against commercial fraud.

Previous studies have employed a range of classic analytical techniques and sensory measurements to establish the quality of vinegar, such as pyrolysis-mass spectrometry (Anklam et al. 1998; Xiong et al. 2017), gas chromatography-olfactometry (Corsini et al. 2019), atomic absorption spectrometry (Ozbek and Akman 2016), the e-nose system (Yin et al. 2017) or symmetrical chemosensors (Suganya et al. 2014). However, these techniques are all expensive and slow and cause pollution.

It would therefore be of great interest and use to the vinegar industry to have a non-destructive technology which is fast, accurate, low-cost and environmentally friendly, and which can be applied directly in the wine cellar, providing real-time data, with the added possibility of its use for establishing the chemical and sensory quality standards of the product analysed.

The combination of speed, precision and low cost makes NIRS one of the most suitable alternative technology to traditional analytical methods for measuring the chemical quality of vinegars (Saiz-Abajo et al. 2006; Bao et al. 2014; De la Haba et al. 2014; Ríos-Reina et al. 2018).

Saiz-Abajo et al. (2004) used NIRS technology to differentiate between wine vinegars (white or red) and alcohol vinegar. Casale et al. (2006) used NIRS technology to classify vinegars according to their aging period, while Shi et al. (2013) predicted the total acid content and classified the vinegars according to the raw material used. De la Haba et al. (2014), whose preliminary results look highly promising, evaluated the potential of NIRS technology as a non-destructive method to characterize wine vinegars belonging to *Vinagre de Montilla-Moriles*

(PDO) and to classify them according to the manufacturing process used. Rios-Reina et al. (2018, 2019) used NIRS technology to authenticate Spanish wine vinegars registered with a PDO and control the authenticity of their commercialized categories.

The objective of this work was to study the industrial application of NIR spectroscopy to measure the quality parameters of wine vinegars belonging to *Vinagre de Montilla-Moriles* (PDO) by analysing the commonest chemical controls performed in the industry, especially in the case of vinegars with different sugar contents (dry, semi-sweet, sweet and balsamic).

Materials and Methods

Reference Samples and Chemical Analysis

A total of 107 white wine vinegars belonging to *Vinagre de Montilla-Moriles* (PDO) were analysed.

Once the vinegars were received in the laboratory, these were stored at 4°C with 85% relative humidity until the following day, when the NIRS and reference analyses were performed. Before carrying out the analytical measurements, the samples were kept at room temperature in order to achieve a stable product temperature of 20°C, the optimum temperature for carrying out the tests.

Once the NIR spectra were taken, the analytical measurements of the parameters to be studied (volumic mass, reducing sugars, total acidity and pH), were developed.

The volumic mass was determined by aerometry (ODEC 1990). Reducing sugars were measured by titration using an automatic titrator (Crison Micro TT 2050, Crison, Alella, Barcelona, Spain) (Rebelein 1973). Total acidity was measured by titration using an automatic titrator (Crison Micro TT 2050, Crison, Alella, Barcelona, Spain) following OENO resolution

52-2000 revised by OIV-OENO 597-2018 (IOV 2018). The pH was measured using potentiometry with an automatic titrator (Crison Micro TT 2050, Crison, Alella, Barcelona, Spain). All the analytical measurements were made in duplicate and the standard error of laboratory (SEL) was calculated from these replicates.

Spectral Data Collection

The NIR spectra of the vinegars were collected in transflectance mode (log (1/R) using a dispersive monochromator-based instrument FNS-6500 SY-I (FOSS NIRSystems, Silver Spring, MD, USA). A folded-transmission gold circular reflector cup, diameter 3.75 cm, with a path length of 0.1 mm was used.

The FNS-6500 SY-I provides absorbance values between 400 and 2500 nm, every 2 nm, covering both the visible and the near infrared region and is equipped with a spinning module that rotates the cup. Two spectra were collected per sample and averaged for subsequent processing.

Data Processing and Development of Predictive Models Using Modified Partial Least Squares Regression

Data pre-processing and chemometric treatments were performed using the WinISI II software package version 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) (ISI, 2000).

Before to carrying out NIRS calibrations, the CENTER algorithm was applied in the spectral range 400-2500 nm to ensure a structured population selection based solely on spectral information, in order to establish the calibration and validation sets (Shenk and Westerhaus, 1991). This algorithm performs an initial principal component analysis to calculate the centre

143 of the population and the distance of samples (spectra) from that centre in an n dimensional
144 space, using the Mahalanobis distance (GH); samples with a GH value > 3 were considered
145 spectral outliers. A combination of mathematical pre-treatments, Standard Normal Variate
146 (SNV) and Detrend (DT) was applied for scatter correction (Barnes et al., 1989), together with
147 the first derivate treatment '1,5,5,1' (the first digit being the number of the derivative, the
148 second the gap over which the derivative is calculated, the third the number of data points in a
149 running average or smoothing, and the fourth the second smoothing) (Shenk and Westerhaus,
150 1995b; ISI, 2000). Once the samples were ordered by distance from the centre of the population,
151 3 out of every 4 samples were selected to be part of the calibration set, while the remaining
152 samples formed the validation set (Table 1).

153 NIRS calibration models for the parameters tested were developed using modified
154 partial least squares (MPLS) regression (Shenk and Westerhaus, 1995a). Four cross-validation
155 steps were included in the process in order to avoid overfitting (Shenk and Westerhaus, 1995a).
156 For scatter correction, SNV and DT methods were applied ~~(Barnes et al., 1989)~~. Additionally,
157 first ('1,5,5,1') and second ('2,5,5,1') derivate treatments were tested (Shenk and Westerhaus,
158 1995b; ISI, 2000). Finally, 2 spectral regions were also studied to develop these models:
159 Vis/NIR: 400-2500 nm and only NIR: 1100-2500 nm.

160 The statistics used to select the best equations were: standard error of calibration (SEC),
161 coefficient of determination for calibration (r^2_c), standard error of cross-validation (SECV),
162 coefficient of determination for cross-validation (r^2_{cv}). In addition, the Residual Predictive
163 Deviation statistic for cross-validation (RPD_{cv}) was calculated as the ratio of the standard
164 deviation of the original data (SD) to SECV. This latter statistic enables SECV to be
165 standardized, facilitating the comparison of the results obtained with sets of different means
166 (Williams 2001).

The SECV values for the best equations obtained for the two spectral ranges selected were compared using Fisher's F test (Massart et al., 1988; Naes et al., 2002). Values for F were calculated as:

$$F = \frac{(SECV_2)^2}{(SECV_1)^2}$$

where $SECV_1$ and $SECV_2$ are the standard error of cross validation of two different models and $SECV_1 < SECV_2$. F is compared to $F_{critical\ (1\text{-}P,\ n1\text{-}1,\ n2\text{-}1)}$, as read from the table, with $P = 0.05$ and n_1 is the number of times the measurement is repeated with method 1, while n_2 is the number of times the measurement is repeated with method 2. If F is higher than $F_{critical}$, the two SECV values are significantly different.

Once the best spectral region of the instrument was selected, the best equations obtained for this region were subjected to an external validation process following the protocol established by Windham et al. (1989).

Results and Discussion

Descriptive Data for NIRS Calibrations and Validations Sets

Values for number of samples, range, mean, standard deviation and coefficient of variation for each of the parameters analysed for the calibration and validation sets obtained after application of the CENTER algorithm (no sample was considered as spectral outlier) are shown in Table 1. Structured selection using only spectral information treatment algorithms such as CENTER proved adequate, since the calibration and validation sets displayed similar values for mean, range and standard deviation for all study parameters, and ranges for the validation set lay within the range recorded for the calibration set.

191 The parameter which presented the greatest variability was the reducing sugars content
192 ($CV_{calibration}$ = 89.66%; $CV_{prediction}$ = 82.65%), since both groups were composed of dry vinegars
193 (with a content in reducing sugars of below 5 g/L), semi-sweet vinegars (5–69 g/L), sweet
194 vinegars (over 70 g/L) and balsamic vinegars with reducing sugar content of over 150 g/L
195 (BOE, 2012; OJEU 2015).
196 +The parameter with the lowest variability was volumic mass ($CV_{calibration}$ = 4.15%; $CV_{prediction}$
197 = 4.28%), since all the vinegars were originated from wines and musts qualified by 'Montilla-
198 Moriles' PDO wine, which mean that all of them had completed the fermentation process.
199 As regards the total acidity parameter, the calibration and validation sets varied between
200 37.53% and 39.57%, respectively. The reason for this was that among the samples analysed,
201 there were vinegars with a content slightly less than 6 g acetic acid/100 mL vinegar, as was the
202 case of sweet vinegars obtained by adding grape musts at different stages of aging; on the other
203 hand, there was another group of samples with a fairly high acetic acid content (14-19 g acetic
204 acid/100 mL vinegar) as a result of prolonged aging in wooden barrels, which causes the water
205 to evaporate and a higher concentration in the acetic acid.
206 The pH parameter has a low variability ($CV_{calibration}$ = 14.70%; $CV_{prediction}$ = 7.45%),
207 albeit greater than the volumic mass. This is because, among the samples analysed, there are
208 vinegars with a high acetic acid content (18.6 g/100 mL) and a consequently low pH (pH =
209 2.40), while, at the other end of the scale, there are vinegars with a high concentration of
210 reducing sugars (over 300 g/L) and low acetic acid content (5.1 g/100 mL) with a pH = 5.77.
211 The pH value is not mentioned in the regulations.
212
213 Construction of Calibration Models for Predicting Chemical Quality parameters in Wine
214 Vinegars of *Vinagre de Montilla-Moriles* (PDO)
215

Table 2 shows the statistics for the best equations obtained for the chemical quality parameters predicted with the NIR instrument, for the two spectral ranges studied.

For the parameter of volumic mass, the models obtained display an excellent predictive capacity, according to Shenk and Westerhaus (1996) and Williams (2001). Likewise, Nicolaï et al. (2007) showed that RPDcv values over 3 confirm that the model has an excellent predictive capacity.

No research articles have been found in the scientific literature related with measuring volumic mass in wine vinegar using NIRS technology. However, it is important to measure this parameter, which is related to the soluble solid content (mainly polyphenols and reducing sugars), in vinegars with a strong colour or a high sugar content, which are characterized by having higher values of this parameter. Incidentally, this parameter is also used in wine cellars to convert weight to volume in both sales and purchases.

As regards the parameter of reducing sugars, if the values of r^2_{cv} and RPD_{cv} are taken into account, the predictive capacity of the models developed can be considered to be excellent, according to the values indicated by Shenk and Westerhaus (1996), Williams (2001) and Nicolaï et al. (2007) for these statistics.

No scientific articles have been published which deal with measuring this parameter in wine vinegar using NIRS technology. However, it is extremely important to measure this parameter in a non-destructive way in the wine cellars when carrying out vinegar qualification. Moreover, currently there is huge interest in Montilla-Moriles (PDO) in the production of sweet vinegars, in order to compete with other leading European producers for its high quality and organoleptic.

In relation to the high values of the coefficient of determination for cross-validation obtained for this parameter, it is important to consider the observations made by Fearn (2014), who noted that, although the r^2_{cv} statistic can be useful in establishing the predictive capacity

241 of a particular model, this statistic has its limitations, mainly that it depends on the range and
242 the standard deviation of the calibration group. In this specific case, the calibration group is
243 made up of dry, semi-sweet and sweet vinegars with different levels of aging; for this reason
244 the standard deviation is practically equal to the average, with a very wide parameter range.
245 As regards the parameter of total acidity, the predictive capacity of the model used is
246 also excellent when considering the values of r^2_{cv} and RPD_{cv} (Shenk and Westerhaus 1996;
247 Williams 2001; Nicolaï et al. 2007).
248 The non-destructive measurement of this parameter is extremely important for the
249 industry: vinegars which stand out for their high total acidity correspond to vinegars with long
250 aging periods, such as Reserve vinegars, where a large amount of water was evaporated.
251 However, there are other vinegars which do not appear to reach the minimum total acidity
252 established by the quality standard for Spanish vinegars (BOE 2012). These low acetic levels
253 are due either to the fact that the acetification process has not finished, or to the recent addition
254 of fresh wine to the barrels, which thereby dilutes its acetic acid content (López et al. 2003).
255 The results obtained are lower than those obtained by De la Haba et al. (2014) (RPD_{cv}
256 = 8.35), using the same monochromator technology with the spinning module. However, it must
257 be highlighted that although the calibration set of those authors displayed a lower variability,
258 the distribution of the samples was more uniform along the range of values for this parameter,
259 which can be appreciated in the frequency histogram of the calibration set.
260 As for the pH parameter, the predictive capacity of the model allows to differentiate
261 between high, medium and low values for this parameter (Shenk and Westerhaus 1996;
262 Williams 2001). If the RPD_{cv} value obtained is taken into account, according to Nicolaï et al.
263 (2007), the model should allow to differentiate between high and low values of this parameter.
264 It is important to note that during the vinegar aging process, the initial pH usually decreases,
265 and that pH values below 3 hinder the development of acetic bacteria. It should also be

266 considered that ‘Pedro Ximénez’ or ‘Moscatel’ musts are added to the sweet vinegars included
267 in this PDO, thus causing the initial pH to rise.

268 The results of the predictive models in this work are similar to those obtained by Bao et
269 al. (2014) and De la Haba et al. (2014).

270 Dardenne (2010) and Fearn (2014) showed that the RPD_{cv} statistic used in most NIR
271 research articles is equal to $1 / \sqrt{(1 - r^2_{cv})}$ and depends to the same extent, as with r^2_{cv}, on the
272 range existing in the calibration set. Here, Table 2 shows the correlation between the high and
273 low r^2_{cv} and RPD_{cv} values for the parameters analysed. It can be seen that for the parameters of
274 volumic mass, reducing sugars and total acidity, models with r^2_{cv} values between 0.95 and 0.99
275 and RPD_{cv} between 4.64 and 14.14 are obtained, while for the pH parameter, values are
276 obtained of $r^2_{cv} = 0.59$ and 0.60 and $RPD_{cv} = 1.50$ and 1.55 for models developed in the spectral
277 regions of 1100 to 2500 nm and 400-2500 nm, respectively.

278 Lastly, the best spectral working region of the FNS-6500 SY-I instrument for the
279 parameters analysed was established. The results of the Fisher’s F tests performed are shown
280 in Table 2. For all the parameters analysed, no significant differences ($P < 0.05$) were detected
281 between the SECV values obtained in the two spectral regions considered. As it would be of
282 interest in the future to incorporate analytical parameters related to the colour of the vinegar, it
283 was decided to work with the full spectral range of the instrument, i.e., 400-2500 nm.

284

285 External Validation

286

287 Table 3 shows the external validation statistics of the best models obtained to predict the
288 chemical quality parameters in white wine vinegars belonging to *Vinagre de Montilla-Moriles*
289 (PDO), in the spectral range between 400 and 2500 nm.

290 Windham et al. (1989) established the conditions which must be met by the models to
291 be used in the routine prediction of quality parameters. Following this protocol, the models
292 constructed for predicting volumic mass met the validation requirements in terms of the
293 coefficient of determination for prediction, r^2_p (r^2_p >0.6), and the standard error of prediction
294 corrected for bias ($SEP_{(c)}$), the bias and the slope were within confidence limits: the models thus
295 ensure accurate prediction and can be applied routinely. For the reducing sugar the r^2_p, bias and
296 slope lay within the confidence limits, however, $SEP_{(c)}$ value did not attain the recommended
297 value. The total acidity parameter has higher value of $SEP_{(c)}$ compared with the control value.
298 In addition, the slope for total acidity (0.85) is lower than that established in the protocol (0.9-
299 1.1) and only r^2_p and bias met the validation requirements. Finally, the pH parameter did not
300 meet any of the four established limits ($SEP_{(c)}$, bias, r^2_p and slope) under this protocol.
301 In addition, comparing the values of the SEL (Table 1) and SEP (Table 3) statistics for
302 each of the parameters analysed, it can be confirmed that the models developed cannot be used
303 routinely because the SEP values exceed more than 5 times the values of the calculated SELs
304 (Westerhaus 1989; Williams 2001).
305 This research constitutes, therefore, an initial approach to the use of NIRS technology
306 for the quality control of wine vinegars of *Vinegar of Montilla-Moriles* (PDO). Larger
307 calibration groups which would reflect the variability of the product, are needed in order to
308 obtain more robust models which can be used routinely.
309
310 Main Wavelengths for Predicting the Quality Parameters Analysed in Wine Vinegars of
311 *Vinagre de Montilla-Moriles* (PDO)
312
313 The loading plots corresponding to the best models obtained for predicting the quality
314 parameters (volumic mass, reducing sugars, total acidity and pH) in white wine vinegars of the

315 *Vinagre de Montilla-Moriles* (PDO) are show in Fig. 1. These plots show the areas across the
316 spectral range where variance has influenced computing of the model to a greater or lesser
317 degree, and the direction (positive or negative).

318 For the parameter of volumic mass, the representation of the first 4 latent variables used
319 in the development of the calibration model showed that the areas of the spectrum which exert
320 the greatest influence on the model fit were 468, 494, 948, 1448, 1664, 1930, 2234, 2282, and
321 2458 nm, which are linked to the presence of anthocyanins and chlorophyll in the visible region
322 and with water and carbohydrates in the near-infrared region (Williams 2001; Shenk et al.
323 2008).

324 For the parameter of reducing sugars, the areas of the spectrum with the greatest
325 influence were: 436, 494, 946, 1436, 1660, 1898, 2130, 2234, 2284, 2332 and 2456 nm, related
326 to pigments (anthocyanins and chlorophyll) in the visible region and with water, carbohydrates
327 and proteins in the NIR region (Williams 2001; Shenk et al. 2008). It is important to note that
328 the wavelengths which influence this parameter also have the same effect in the case of the
329 parameter of volumic mass.

330 For the parameter of total acidity, the main wavelengths detected were: 476, 634, 926,
331 1382, 1876, 2042, 2266 and 2458 nm, related to orange and green pigments and water,
332 carbohydrates and proteins (Williams 2001; Shenk et al. 2008), very similar to those of pH,
333 since these two parameters are closely related.

334

335 **Conclusions**

336

337 The results obtained show that NIR spectroscopy, combined with suitable chemometric
338 methods, could be used to measure volumic mass, reducing sugars and total acidity in vinegar.
339 These parameters are of great importance in monitoring the fermentation process of this

product, as well as in detecting commercial fraud in the vinegar industries. These measurements will facilitate real-time decision-making throughout the production process and when the vinegars are later aged. However, the results obtained should be considered as preliminary, being necessary in the future to increase the robustness of the models obtained, thus permitting the routine use of this technology in the vinegar industries.

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Compliance with Ethical Standards:

Conflict of Interest

María-Teresa Sánchez declares that she has no conflict of interest. Rocío Márquez declares that she has no conflict of interest. Irina Torres declares that she has no conflict of interest. María-José de la Haba declares that she has no conflict of interest. Dolores Pérez-Marín declares that she has no conflict of interest. María-Isabel López declares that she has no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Informed consent was obtained from all individual participants included in this study.

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494 **Table 1** Calibration and validation sample sets and standard error of laboratory

Parameter	Statistics	Calibration set	Validation set
Volumic mass (g/L)	N[a]	80	27
	Range	1012-1137	1013-1126
	Mean	1063.10	1065.11
	SD[b]	44.16	45.61
	CV[c] (%)	4.15	4.28
	SEL[d]	0.27	
Reducing sugars (g/L)	N	80	27
	Range	8.25-358.5	16.30-314.45
	Mean	132.64	147.72
	SD	118.92	122.09
	CV (%)	89.66	82.65
	SEL	1.26	
Total acidity (g acetic acid/100 mL vinegar)	N	80	27
	Range	4.20-19.0	5.05-17.1
	Mean	7.70	7.53
	SD	2.89	2.98
	CV (%)	37.53	39.57
	SEL	0.10	
pH	N	80	27
	Range	2.40-5.77	2.54-3.36
	Mean	2.79	2.82
	SD	0.41	0.21
	CV (%)	14.70	7.45
	SEL	0.01	

495 [a] Number of samples

496 [b] Standard deviation

497 [c] Coefficient of variation

498 [d] Standard error of laboratory

499 **Table 2** Calibration statistics of the best models obtained for the prediction of chemical quality

500 in wine vinegars belonging to *Vinagre de Montilla Moriles* (PDO)

Parameter	Spectral range (nm)	Math treatment	N[a]	Range	SEC[b]	r^2_c[c]	SECV[d]	r^2_{cv}[e]	RPD_{cv}[f]	F	$F_{critical}$
Volumic mass (g/L)	400-2500	2,5,5,1	73	1112-1137	3.66	0.99	4.09	0.99	10.69	1.10	1.47
	1100-2500	1,5,5,1	74	1112-1137	3.99	0.99	4.30	0.99	10.11		
Reducing sugars (g/L)	400-2500	2,5,5,1	72	8.25-332.50	7.25	0.99	8.03	0.99	14.05	1.01	1.48
	1100-2500	1,5,5,1	72	8.25-332.50	7.32	0.99	7.98	0.99	14.14		
Total acidity (g acetic acid/ 100 mL vinegar)	400-2500	1,5,5,1	74	4.20-12.60	0.38	0.97	0.50	0.95	4.50	1.13	1.47
	1100-2500	2,5,5,1	73	4.20-12.60	0.31	0.98	0.47	0.95	4.64		
pH	400-2500	1,5,5,1	72	2.43-3.10	0.10	0.68	0.11	0.60	1.55	1.19	1.47
	1100-2500	1,5,5,1	74	2.43-3.25	0.11	0.66	0.12	0.59	1.50		

501 [a] Number of samples.

502 [b] Standard error of calibration.

503 [c] Coefficient of determination of calibration.

504 [d] Standard error of cross-validation.

505 [e] Coefficient of determination of cross-validation.

506 [f] Residual predictive deviation for cross-validation.

507

508 **Table 3** Validation statistics for the best equations for the prediction of chemical quality for

509 *Vinagre Montilla-Moriles* (PDO). Spectral range 400-2500 nm

Parameter	N [a]	SEP [b]	$SEP_{(c)}$ [c]	Bias	r^2_p [d]	Slope	Limits [e]	
							$SEP_{(c)} = 1,3 \cdot SEC$	Bias = ± 0,6 · SEC
Volumic mass (g/L)	27	3.23	2.96	-1.42	0.99	1.00	4.76	± 2.20
Reducing sugars (g/L)	27	13.97	14.17	1.31	0.99	1.01	9.43	± 4.35
Total acidity (g acetic acid/100 mL vinegar)	27	1.42	1.43	-0.20	0.80	0.85	0.49	± 0.23
pH	27	0.22	0.20	0.11	0.22	0.75	0.13	± 0.06

510 [a] Number of samples for the validation set.

511 [b] Standard error of prediction.

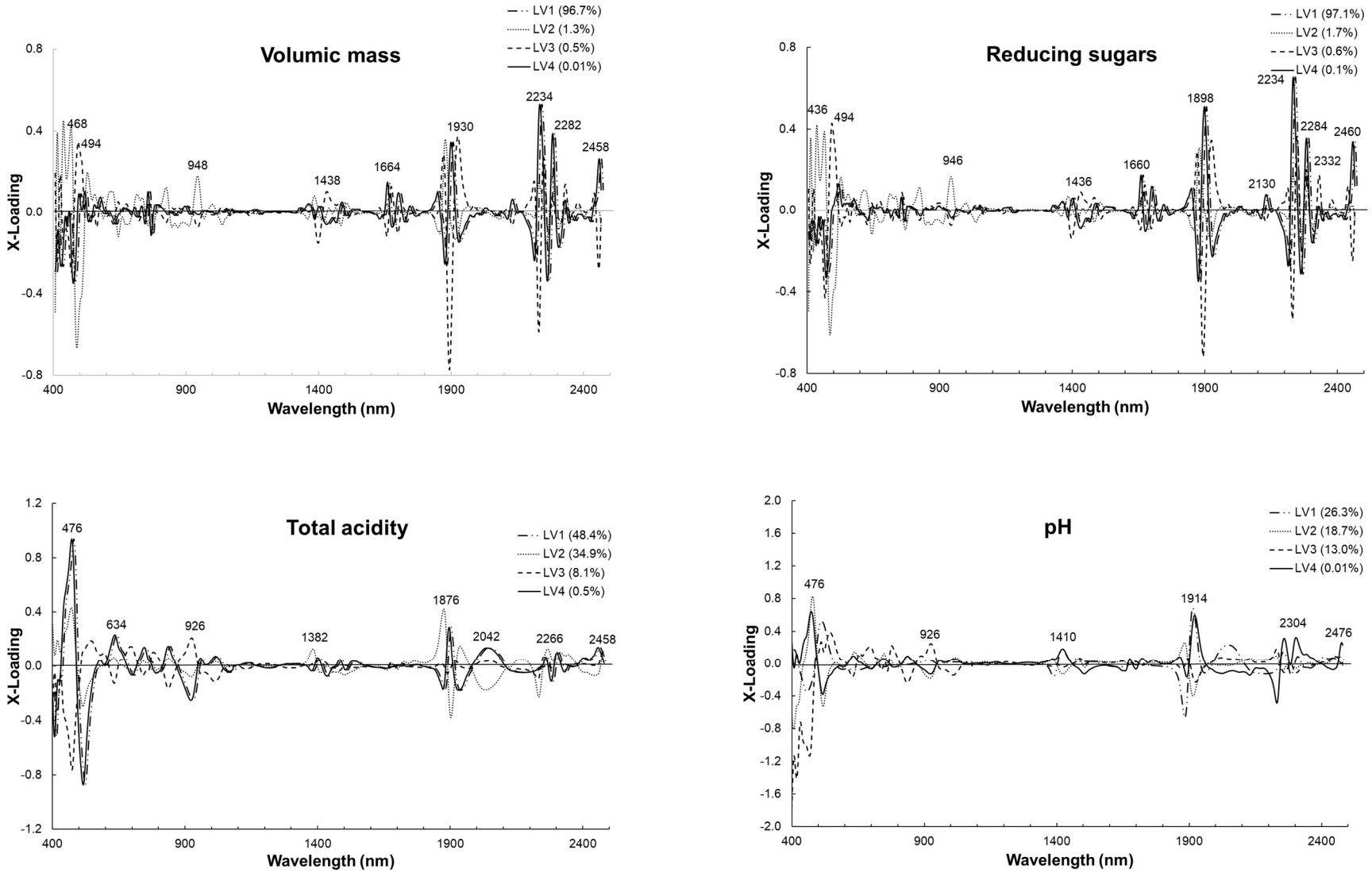
512 [c] Standard error of prediction bias-corrected.

513 [d] Coefficient of determination of prediction

514 [e] Control limits established in the protocol of Windham et al. (1989)

515

516 **Fig. 1** Loadings for the parameters volumic mass. reducing sugars. total acidity and pH



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