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Abstract: There is substantial variation in levels of extractable phenolic compounds of red grapes (Vitis vinifera L.). Therefore, it could be desirable to known the aforesaid parameter at least for each vine. Nowadays, interest has shifted toward the development of portable vis/NIR systems, innovation in optical system design and miniaturization for its friendly use directly in the field.
Spectra of intact grapes and grapes skins were recorded at harvest time in two different vintages (2016 and 2017 respectively) using a portable micro NIR spectrophotometer (908-1676 nm). A number of chemometric approaches have been used for spectral interrogation and evaluation of the aforesaid device. Spectral data have been correlated with red grape skin extractable polyphenols (total phenolic, anthocyanins and flavanols) by modified partial least squares regression (MPLS) using a number of spectral pretreatments. Moreover, different statistics strategies have been performed to develop a qualitative analysis of the data (linear discriminant analysis, discriminant partial least square analyses and Pearson's similarity index).
After an exhaustive analysis of the obtained results in two different seasons, it can be concluded that the use of the portable micro NIR device for the "in vineyard" screening of extractable polyphenols in red grape skins is hampered by a number of factors. Environmental and physiological conditions should be considered to evaluate and remove factors that hamper a good sorting the berries according to their extractable polyphenol contents.

Highlights

- Extractable polyphenols from red grape skin have been studied in two seasons
- Recording of grapes spectral data using portable NIR spectrometer has been achieved
- Calibration MPLS models have been developed from reference and spectral data
- Qualitative approaches have been tested for the extractable polyphenols prediction
- Due to environmental and physiological conditions, models show poor results

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Feasibility study on the use of a portable micro near infrared spectroscopy device for the "*in vineyard*" screening of extractable polyphenols in red grape skins

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ABSTRACT

There is substantial variation in levels of extractable phenolic compounds of red grapes (*Vitis vinifera* L.). Therefore, it could be desirable to known the aforesaid parameter at least for each vine. Nowadays, interest has shifted toward the development of portable vis/NIR systems, innovation in optical system design and miniaturization for its friendly use directly in the field.

Spectra of intact grapes and grapes skins were recorded at harvest time in two different vintages (2016 and 2017 respectively) using a portable micro NIR spectrophotometer (908-1676 nm). A number of chemometric approaches have been used for spectral interrogation and evaluation of the aforesaid device. Spectral data have been correlated with red grape skin extractable polyphenols (total phenolic, anthocyanins and flavanols) by modified partial least squares regression (MPLS) using a number of spectral pretreatments. Moreover, different statistics strategies have been performed to develop a qualitative analysis of the data (linear discriminant analysis, discriminant partial least square analyses and Pearson's similarity index).

After an exhaustive analysis of the obtained results in two different seasons, it can be concluded that the use of the portable micro NIR device for the "*in vineyard*" screening of extractable polyphenols in red grape skins is hampered by a number of factors. Environmental and physiological conditions should be considered to evaluate and remove factors that hamper a good sorting the berries according to their extractable polyphenol contents.

Keywords: extractable polyphenols, red grapes, portable spectroscopy, NIR, chemometrics, wine

Introduction

Red grapes (*Vitis vinifera* L.) contain about four grams of phenolic material per kilo. These compounds are secondary metabolites that play crucial roles in the plant kingdom. There are substantial variations in levels of phenolic compounds which depends on a number of physiologic, agronomic or climatological factors [1, 2]. Wine and grape phenolic compounds are grouped into two categories, flavonoids and non-flavonoids. Wine flavonoids are all polyphenolic compounds, having multiple aromatic rings presenting hydroxyl groups [3]. Flavonoids have well-known health benefits. They possess ideal structural chemistry for free radical-scavenging activities, and they have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis [4].

Most flavonoids in red grapes are found in berry solid parts and they are transferred to the wine during the fermentation process. Wine flavonoids (mainly flavonols, flavanols and anthocyanins) play a relevant role in the sensory characteristic of red wines. They are directly or indirectly responsible for wine color [3, 5, 6] and have a strong influence in wine taste (astringency, sourness, bitterness, etc.) [7].

Taking into account these aspects, it could be desirable to know the amount of these phenols that may be extracted from grapes to wine, at least for each vine. The conventional chemical methods used for determination of these parameters are destructive and time consuming because they require the extractions of different phenols from grape skin using wine simulated macerations [8-10]. Near infrared (NIR) spectroscopy has been widely used in the oenological field for grape and wine analysis [11]. This technique has shown considerable potential for the nondestructive determination of the main families of phenolic compounds in grapes [12, 13]. The use of NIR spectroscopy to predict total soluble solids, pH, and total anthocyanins in red

grapes [14, 15] and other technological parameters useful for classifying grapes [16] have been also reported. In a further step, near infrared hyperspectral imaging has been used to develop screening methods to measure total or extractable phenols in grapes [17, 18]. This methodology may allow sorting the berries according to their extractable polyphenol contents and then the same samples could be used in further studies for other destructive analyses or purposes. However, despite the fast and effective proficiency of near-infrared spectroscopy to predict different parameters in wine sector, most of these studies carried out at lab imply sample transportation. To solve this problem portable hand-held NIR spectrometers have been recently used to acquire NIR spectra in vineyards, directly on-the-vine [19-22]. However, it should be taken into account that these portable systems are composed of different elements such lightening system, batteries and fiber optic probe in addition to the portable spectrophotometer that may difficult their used in field conditions. In a further step, interest has shifted toward the development of portable vis/NIR systems using Linear Variable Filter (LVF), innovation in optical system design and miniaturization due to the fact that it does not need any external components because all the needed parts are incorporated into its design. Although limited information is still available with regards to this technology on the enology sector, their use could be significantly hindered by the varying conditions of field measurements.

A huge amount of information generated by all these spectroscopic devices has to be correctly processed to obtain useful information. Quantitative or qualitative chemometric tools are usually applied for the development of calibration or classification methods. Partial least square (PLS) regression has been widely used for the development of calibration methods for the prediction of different parameters in grapes [23]. Moreover, supervised pattern recognition methods, such as discriminant

partial least square (DPLS) analysis or linear discriminant analysis (LDA), are usually applied to the identification of spatial regions of interest in oenological samples [24, 25] or to the classification of grape samples according to some important attribute [26-29].

The main aim of this work is to study the feasibility of the use of a portable micro near infrared spectroscopy device for the "in vineyard" screening of extractable polyphenols in red grape skins. The aforesaid new device does not need any external probes, fiber optics or external illumination sources because all the needed parts are incorporated into its miniaturized design. Grape skin spectra have been collected in two different seasons using two different measurement methodologies to obtain the spectral data. A number of samples spectrally representative have been selected and the extractable contents of total phenols, flavanols and anthocyanins have been chemically evaluated. Finally, different chemometric quantitative and qualitative tools have been interrogated to obtain the best approach for the spectral screening of these extractable contents in grape skin. To the best of our knowledge, this is also the first time that the aforementioned parameters have been jointly evaluated using a portable device.

Material and methods

Samples

Vitis vinifera L. cv. Tempranillo and Syrah red grapes samples from two vineyards located in the Condado de Huelva Designation of Origin D.O. (Andalusia, Spain) were used in the present study. Both varieties are typically grown in Spain for producing quality red wines and being a resistant cultivar to warm climatic conditions [30].

In an attempt to optimize the spectra acquisition procedure, it was designed a systematic experiment which was divided into two seasons as shown in the Fig. 1. To face this task, grapes were collected in two different vintages (2016 and 2017) at harvest time. In

both years NIR spectroscopy analysis was carried out "*in vineyard"* but a modification of measurement conditions was performed.

- In 2016 season grape spectra were collected directly on the bunch, without any sample preparation. Samples whose spectra were recorded were then collected for chemical analysis, to provide reference values for the measured properties.

- In 2017 season the engaging grapes were picked from the bunch and just after that grape skins were manually separated from the whole grapes and placed at the bottom of quartz cuvettes to collect the spectra. Samples were soft-pressed inside the cuvette to increase the contact surface. Spectra were recorded from the external surfaces of the skins.

With the aim of achieving representative sample sets, the grapes were selected from the top, middle and bottom of the bunch and in the sunlight and shade side of vines located in different rows within the vineyard. Edge rows and the first two vines in a row were avoided. A total of 200 spectra were collected in each season, 100 Tempranillo spectra and 100 Syrah spectra.

Once the spectrum was registered, the samples (whole grapes from 2016 and grapes skins from 2017) were placed in stoppered plastics bags, labelled, refrigerated at 4 °C and immediately carried to the laboratory. Therefore, in this study, a total of 400 grape samples have been taken into account (200 samples in 2016 and 200 samples in 2017, i.e. 100 samples per variety and season). Upon arrival at the lab, grapes were frozen and stored at -20 ºC until analyses were performed. Prior to each chemical measurement, grape skins belonging to vintage 2016 were separated manually from the whole grapes and they were weighted. All samples were allowed to stabilize at laboratory temperature (25 °C) before the chemical analysis.

Spectral data acquisition

In-situ acquisition of spectra was performed using a portable NIR spectrophotometer (MicroNIR Pro Lite 1700, VIAVI, Santa Rosa, California, USA), an instrument designed to measure diffuse reflectance in the NIR region of the electromagnetic spectrum. The MicroNIR owes its small size to the novel thin-film linearly variable filter (LVF) used as the dispersive element. The LVF is directly coupled to a linear detector array (128-pixel uncooled InGaAs photodiode array), covering the spectral range between 908 and 1676 nm (spectral resolution of 6.2 nm). The filter coating in the LVF is wedged in one direction and as a result of the varying film thickness; the wavelength transmitted through the filter varies linearly in the direction of the wedge. The LVF makes each pixel of the detector respond to a different wavelength. This ultra-compact spectroscopic engine is coupled with a tungsten lamps diffuse illumination system. An illustration of the MicroNIR spectrometer and MicroNIR´s optical designed, adapted from VIAVI user manual, is provided in Fig. S1.

Spectra were recorded using the instrument acquisition software MicroNIR™ Pro v.2.2 (VIAVI, Santa Rosa, California, USA). A two-point reflectance calibration was used. A Spectralon® ceramic tile was used as a white reference (100% reflectance), whereas dark current (0% reflectance) was recorded by taking a measurement placing the device about 0.5 meters from any object. Because measurements were made on the vineyard, sample temperature was not controlled beforehand; mean temperature on measurement days ranged from 30 to 35 °C, typical extreme temperature of warm climates in August and September. Spectral acquisition was performed in shade using a light-tight box.

Reference parameters

Reference parameters taken into account were extractable total phenolic content, extractable flavanol content and extractable anthocyanin content in grape skin (EPC,

EFC and EAC respectively). To perform these determinations, grape skins were immersed in a model wine hydroalcoholic solution (4 gL^{-1} tartaric acid, 12.5% ethanol, adjusted at pH 3.6 with NaOH 0.5 M) for a maceration period of 72 h. Grape skins were added to extraction media in a 1:20 ratio. Then, supernatants were used into the subsequent analyses.

Extractable total phenolic content was determined using the Folin–Ciocalteu method [31]. Gallic acid was used as a standard for construction of the calibration curve and the concentration of total phenols was expressed as gallic acid equivalent in mg g^{-1} of grape skin.

Extractable flavanol content was determined following a modification of Vivas et al. [32]. Twenty microliters of model wine extractions were mixed with 180 μL of methanol respectively and 1 mL of DMACA reagent. The DMACA (4-dimethylaminocinnamaldehyde) reagent was prepared immediately before use, containing 0.1% (w/v) DMACA in a mixture of HCl:methanol (1:10, v/v). After a ten-minute period, the absorbance at 640 nm was measured for each sample. A calibration curve of (+)–catechin was used for quantification and results were expressed as (+)–catechin equivalent in mg g^{-1} of grape skin.

Both Folin–Ciocalteu and DMACA analyses were performed on an Agilent 8453 UV–visible spectrophotometer (Palo Alto, USA), equipped with diode array detection (DAD). The extract volumes were appropriately modified for samples which needed it.

Extractable anthocyanin content was determined by means of chromatographic analysis following a modification of the method of García-Marino et al. [33] as described elsewhere in Hernández-Hierro et al. [17]. Model wine extractions were diluted 1:2 with 0.1 M HCl, filtered through 0.45 μm pore size filters and directly injected into the

chromatographic system. Results were expressed as mg of malvidin-3-*O*-glucoside equivalents per gram of grape skin.

Chemometric analysis

Quantitative analysis

Before the quantitative analysis, principal component analysis (PCA) was used as unsupervised pattern recognition technique to get information about the latent structure of spectral matrix. This method provides not only information related to spectral outliers and the distribution of samples in the newly-created space, but it is also an important source of knowledge with which to create cross-validation groups used in the calibration process [34, 35]. PCA was also used to select representative samples from the spectral data set. Mahalanobis distances (H) for each sample were calculated and samples were grouped according their neighborhood H values (NH).

For each season, using the raw spectral data, testing different spectral pretreatments and allocating the corresponding EPC, EFC and EAC to each sample, calibrations were performed by modified partial least squares regression (MPLS). In this method, the group of calibration samples is divided into a series of subsets to perform cross-validation to set the number of PLS factors, reduce the possibility of overfitting [35] and remove chemical outliers. Using the $T \geq 2.5$ criterion, samples that presented a high residual value when they were predicted were eliminated from the set.

The software used was Win ISI® (v1.50) (Infrasoft International, LLC, Port. Matilda, PA, USA). This software allowed the data pretreatment, principal components analysis and sample selection and development of quantitative models.

Qualitative approaches

For each reference parameter, grape samples allocated into the calibration and validation sets were respectively split in two different classes according to their

extractable content. The statistical median value of each reference parameter in the calibration set was used to develop these classifications. In this way, samples were identified as samples with low or high EPC, EFC or EAC. These new categorical variables were used in conjunction with spectral data for the development of different qualitative chemometric methods. These methods usually indicate whether samples fall into pre-defined classes, how well, and what causes this separation.

Qualitative analyses were carried out for each reference parameter and season. Linear discriminant analysis (LDA) and discriminant partial least square analysis (DPLS) were applied to the spectral matrixes in order to develop different classification methods. The objective of developing these methods is to obtain fast tools for the classification of grape samples according their extractable contents of phenolic compounds (EPC, EFC or EAC). LDA was carried out via SPSS 22.0 for Windows software package (SPSS, Inc., Chicago, IL, USA). Prior probabilities of classification were used in this analysis taking into account each class size. The prediction ability was estimated considering the percentage of samples correctly classified by the rules developed with the training set using an internal validation procedure and the external validation set available. The variables used were all the scores of the PCs used in the sample selection for each season.

Moreover, DPLS were also carried out. Essentially, a PLS method attempts to concentrate the relevant information contained in the variables measured in a lower number of variables without losing of relevant information. Regression is carried out with these new variables, simplifying the calibration model and interpretation of the results. Win ISI® (v1.50) (Infrasoft International, LLC, Port. Matilda, PA, USA) was used for carried out DPLS analyses and they were performed using, as independent variables (X), grape skin spectra allocated into the calibration sets. In addition,

developed models were tested with spectra allocated into the validation sets and the percentages of samples correctly classified in external validation were obtained.

Pearson's similarity index was also applied to discriminate spectral samples according to their extractable contents. Average spectra of samples allocated into the calibration set with low or high extractable content were respectively obtained. Next, a Pearson's linear regression was performed between each spectral sample and the average spectrum of low extractable content class and the same procedure was repeated for the high class. Following, the Pearson's similarity indexes were calculated as:

$$Similarity\ index = \frac{1}{1 + R^2}$$

Indexes were compared and samples were classified according this procedure in the group that has obtained the higher index (i.e. low or high extractable content), obtaining the percentages of samples correctly classified in internal validation. Last, validation set was used to obtain the percentages of samples correctly classified in external validation. This procedure was repeated for each reference parameter (EPC, EFC and EAC) and for each season and it was carried out via Win ISI® (v1.50) (Infrasoft International, LLC, Port. Matilda, PA, USA).

Finally, percentages of samples correctly classified obtained in each qualitative chemometric analysis were jointly plotted in ROC curves via SPSS 22.0 for Windows software package (SPSS, Inc., Chicago, IL, USA).

Results and discussion

Sample selection

Sample selection was made to reduce the number of samples maintaining as much spectral variety as possible as described elsewhere in Nogales-Bueno et al. [18]. The selection was carried out from a PCA. Using all spectral samples and SNV 2,5,5,1 pretreatment, three and five principal components were taken into account in 2016 and

2017 season respectively. More than ninety five per cent of the spectral variability of original spectral matrix was explained in both cases. Ten and eight spectral outliers (H>3) were found respectively and removed from each spectral matrix respectively. In these new three-dimensional and penta-dimensional spaces created, the samples were grouped according their neighborhood H values (NH). One sample from every group was allocated in the calibration set. In addition, to create the validation set, another sample from every group was selected. So, 32 samples were selected to develop a calibration process in 2016 season. Only 27 samples were allocated in the validation set because some groups created had not more than one sample. Following a similar procedure in 2017, 23 and 18 samples formed the calibration and validation set respectively.

Quantitative calibrations

Quantitative calibrations were developed by modified partial least squares (MPLS) regression. These calibrations were performed using, as independent variables (X) the grape skin spectra allocated into the calibration sets (i.e., 32 and 23 spectral samples for 2016 and 2017 seasons respectively). Reference parameters (EPC, EFC and EAC) previously determined for grape skin samples in each season were used as dependent variables (Y). The statistical parameters of the final calibration equations are shown in Table 1 where N is the number of samples used to obtain the calibration equation after removing samples for chemical reasons (T criterion). The mathematical treatment applied (i.e., the best of the different tried treatment), the range of application, and standard deviations are also shown for each reference parameter.

External validations were carried out for each selected model. In 2016, two samples presented reference values outside of the applicability range of the obtained model in the case of EAC. In 2017, one sample presented reference values outside of the

applicability range of the obtained models in the cases of EPC and EAC. These samples were removed from their respective validation sets and the validation procedures were carried out taking into account only samples which presented reference values within the applicability range of the obtained models. As result of the external validation, the standard errors of prediction (SEP) were obtained for each reference variable, these values were also included in Table 1.

Comparatively, these errors are higher than those obtained in our previous study developed using a bench top instrument [36] but in accordance with the high errors previously obtained by Guidetti et al. [20] for the estimation of extractable anthocyanins and polyphenols in grapes using a portable device. With regard to the aforesaid bench top study, near infrared hyperspectral imaging, with a similar InGaAs sensor, was used for the prediction of the same reference parameters. Hyperspectral imaging was applied to similar samples than ones used in the present study, that is, Syrah and Tempranillo grapes collected in the same region at harvest time and the whole grapes were used for the spectral data acquisition. Moreover, a similar chemometric methodology was applied. Therefore, it is proven that the methodologies applied here, the measurement of whole grapes or grape skins in field with the MicroNIR system, is not as efficient as the in-lab hyperspectral methodology applied in our previous study.

In a further step, other qualitative approaches have been carried out to link the phenolic extractable contents in grape skins to their spectral features in the near infrared region.

Qualitative analysis

For qualitative analysis, the calibration sets of samples are the same described above for the quantitative one. Calibration sets were used to develop internal validations and the validation sets described above were used to develop external validations in this qualitative approach. As results of the qualitative analyses carried out (LDA, DPLS and

Pearson's similarity index), different models for the prediction the extractable content level of phenolic, flavanolic and anthocyanic compounds (EPC, EFC and EAC) were obtained. The percentages of samples correctly classified in internal and external validation for each reference parameter and season are shown in Table 2.

Similar to quantitative results, the different approaches carried out for the discriminations of samples according to their level of extractable compounds show unremarkable results. Only internal validations in 2017 season show fairly good results (especially for LDA). However, these results are not consistent in the external validation procedure and percentages of correctly classified samples fall even more than in 2016 season. To easily compare the different chemometric tools applied, ROC (Receiver Operating Characteristic) curves have been plotted (Fig. 2).

ROC curves confirm the trends deduced from Table 2. The measurement of grape skin in quartz cuvettes, carried out in 2017 season, resulted in a slight improvement in the percentages of samples correctly classified according their EPC, EFC and EAC levels. However, this improvement does not seem to be enough for taking into account these models as useful ones.

Discussion

The influence of different error sources related to the varying conditions of field measurements should be considered. The environmental conditions of the vineyard (extreme temperature conditions in most of cases in a warm climate) maybe played a critical role on the obtained results. This factor is also a critical one not only in portable devices, but also for the benchtop ones. Although the sample collections were carried out early in the morning, there is an important gap between the initial and final temperatures in the same collection session. Moreover, other factors that directly affect the performance of the spectroscopic system such as the berry size variation, the

minimum number of berry samples to build the model, the heterogeneous phenolic distribution inside the berry or the range of the parameter to be assessed could influence the obtained results. The size and geometry of grapes combined with their low spectral reflectance can also be the factors responsible of these results. Grapes are small and spherical samples and therefore, they have a high curvature. Small size differences in the grapes can produce large differences in their curvature and, in consequence, in the reflectance that the MicroNiR device can measure. These might be the causes of the results obtained in 2016 season.

As mentioned above, to reduce this problem, a new measurement methodology was carried out in 2017 season. Grapes skins were placed at the bottom of quartz cuvettes for the spectra acquisition trying to minimize the differences in grape curvature. New models showed better results, although they were not good enough for considering them useful models. The different thickness of the grape skins samples might be contribute to as new source of these errors, especially when bare skins of reduced number of grapes are used as sample. It is well known that NIR radiation penetrates a millimeter or so into the sample, thus, differences in the thickness of the skins should have some influence on the collected spectra. In consequence, grapes, unlike other bigger or grounded samples, do not seem to be susceptible of being correctly measured by portable NIR spectroscopes such as the described in this study.

Conclusion

A number of spectral pretreatments and MPLS calibrations were interrogated to develop quantitative models. Moreover, different chemometric strategies were performed to develop a qualitative analysis of the data. However, the procedure reported here does not present enough accuracy for the "*in vineyard*" screening of extractable polyphenols in red grape skins, although promising results have been obtained in our lab for other

matrix [37] and for the same matrix using a similar benchtop methodology [18]. Although the aforesaid device has been developed for its use out of lab, vineyard environmental conditions (extreme temperature conditions in most of cases in a warm climate) maybe play a critical role on its use. This factor is also a critical one not only in portable devices, but also for the benchtop ones. Furthermore, heterogeneity of analyzed grapes and the own features of the berries (size, geometry or skin grape thickness) may also have influence on the obtained data and especial attention should be paid for further studies.

Abbreviations used

NIR, near infrared; MPLS, modified partial least squares; LVF, linear variable filter; PLS, partial least squares; DPLS, discriminant partial least square; LDA, linear discriminant analysis; EPC, extractable total phenolic content; EFC extractable flavanol content; EAC, extractable anthocyanin content; DMACA, 4-dimethylaminocinnamaldehyde; DAD, diode array detector; PCA, principal component analysis; H, Mahalanobis distance; NH, neighborhood Mahalanobis distance; ROC, receiver operating characteristic; SEP, standard error of prediction.

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Figure captions[1]

Fig.1. Schematic representation of the experimental design.

Fig.2. Receiver operating characteristic (ROC) curves of different chemometric tools applied (LDA, DPLS and Pearson's similarity index) for each parameter (extractable total phenolic content, extractable flavanol content and extractable anthocyanin content) and each season (2016 and 2017). Internal and external validation results are shown.

1 NOTE: All figures should be in color on the Web and in black-and-white in print.

Table 1. Main statistical descriptors for the MPLS models developed in the NIR zone close to 908-1676 nm in 2016 and 2017 seasons.

Season	Spectral pretreatments	Reference Parameters	T outliers	PLS factors	N[a]	Est. Min	SD[b]	Est. Max	SEC[c]	RSQ[d]	SECV[e]	SEP[f]
2016	None 2,7,7,1	EPC[g]	1	2	31	0.39	1.82	11.29	1.62	0.20	1.72	2.66
	MSC 2,5,5,1	EFC[h]	1	2	31	0.00	0.50	2.57	0.42	0.32	0.49	0.60
	Detrend 2,13,13,1	EAC[i]	2	4	30	0.00	0.47	2.15	0.33	0.49	0.43	0.43
2017	Detrend 1,5,5,1	EPC[g]	0	2	23	0.00	3.45	18.71	3.04	0.22	3.41	4.04
	MSC 2,5,5,1	EFC[h]	0	5	23	0.00	0.51	2.66	0.28	0.70	0.44	0.64
	SNV 0,0,1,1	EAC[i]	0	2	23	0.00	0.44	2.48	0.34	0.42	0.36	0.60

[a]N: number of samples (calibration set); [b]SD: standard deviation; [c]SEC: standard error of calibration; [d]RSQ: coefficient of determination (calibration set); [e]SECV: standard error of cross-validation (2016: 7 cross-validation groups; 2017: 8 cross-validation groups); [f]SEP: standard error of prediction (external validation); [g]EPC: extractable total phenolic content (mg g^{-1} of grape skin, expressed as gallic acid equivalents); [h]EFC: extractable flavanol content (mg g^{-1} of grape skin, expressed as catechin equivalents); [i]EAC: extractable anthocyanin content (mg g^{-1} of grape skin, expressed as gallic acid equivalents).

Table 2. Percentages of samples correctly classified as samples with low or high extractable contents of total phenols, flavanols and anthocyanins in seasons 2016 and 2017 by different chemometric tools.

Season	Chemometric tool	EPC[a]		EFC[b]		EAC[c]	
		Internal (%)	External (%)	Internal (%)	External (%)	Internal (%)	External (%)
2016	LDA[d]	65.6	48.1	68.8	59.3	59.4	55.6
	DPLS[e]	56.3	40.7	75.0	40.7	75.0	48.1
	Pearson[f]	68.8	59.3	46.9	37.0	59.0	59.0
2017	LDA[d]	87.0	44.4	91.3	44.4	91.3	33.3
	DPLS[e]	69.6	50.0	87.0	55.6	78.3	66.7
	Pearson[f]	73.9	50.0	56.5	72.2	73.9	61.1

[a]EPC: extractable phenolic content; [b]EFC: extractable flavanol content; [c]EAC: extractable anthocyanin content; [d]LDA: linear discriminant analysis; [e]DPLS: discriminant partial least square; [f]Pearson: Pearson's similarity index.

*Graphical Abstract (for review)

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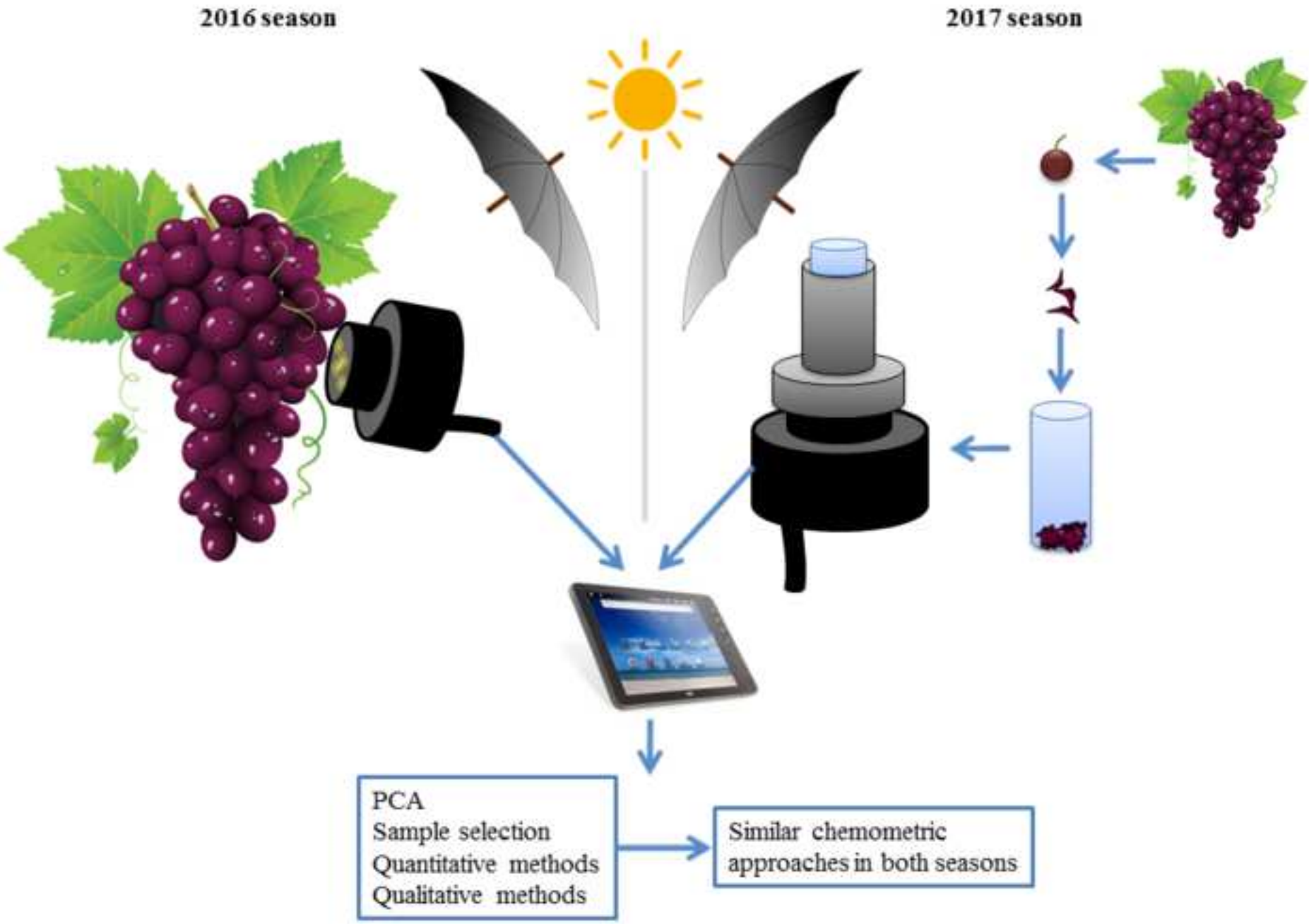

Figure 2

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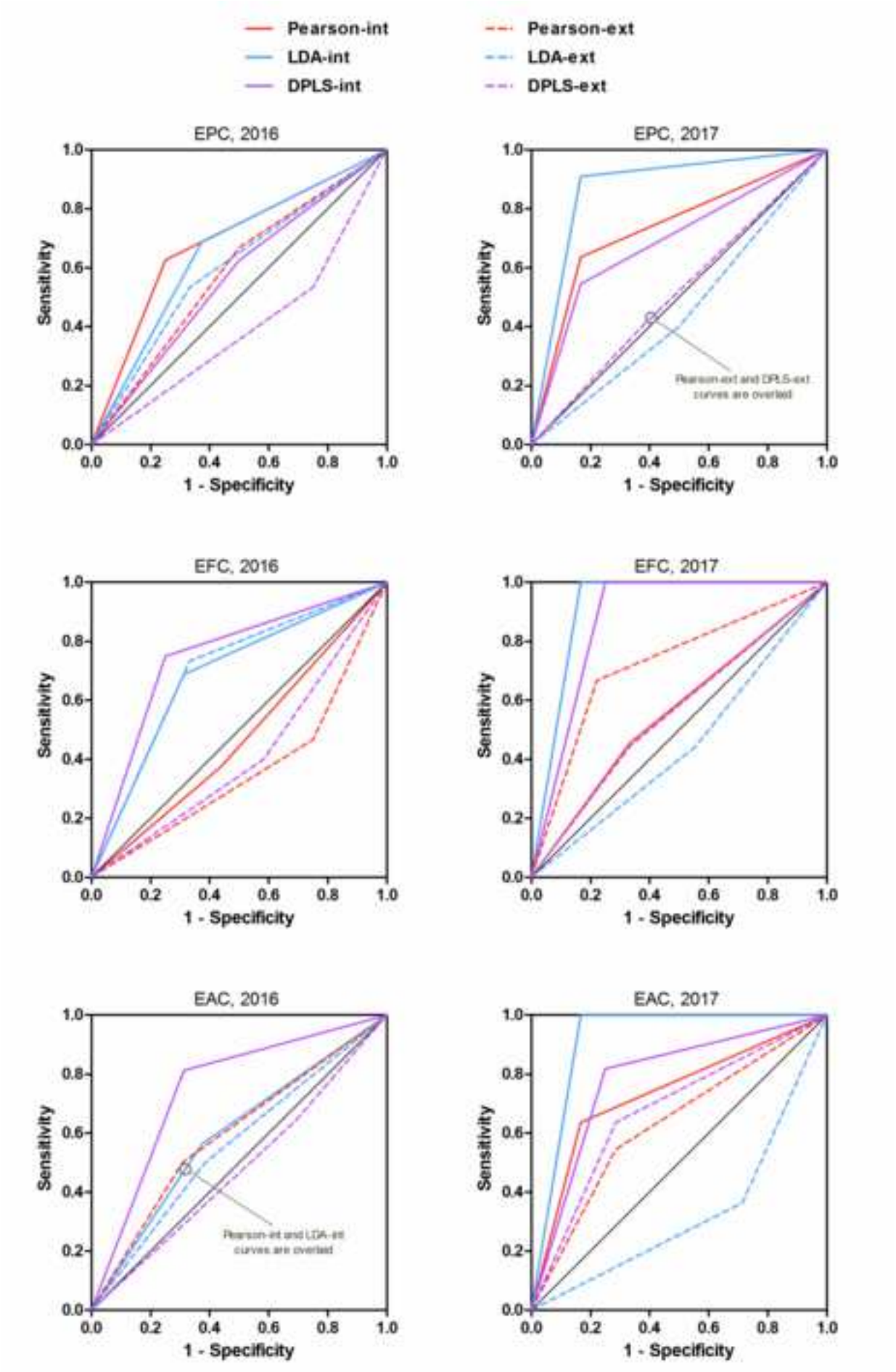

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