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Simultaneous determination of sorbic and benzoic acids in commercial juices using the PLS-2 multivariate calibration method and validation by high performance liquid chromatography

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Abstract

A new method to determine a mixture for preserving sorbic and benzoic acids in commercial juices is proposed. The PLS-2 model was obtained preparing 40 standard solutions adding concentration of sorbic and benzoic acid to filtered natural juices of apple, lemon, orange and grapefruit. The concentration of analytes in the commercial samples was evaluated using the obtained model by UV spectral data. The PLS-2 method was validated by high performance liquid chromatography (HPLC), finding a relative error less than 12% between the PLS-2 and HPLC methods in all cases.

Keywords: Preservings; Juices; Benzoic and sorbic acids; PLS-2 method; Multivariate calibration; HPLC

1. Introduction

Due to the fact that the simultaneous determination of diverse analytes concentration with similar spectral characteristics is almost impossible by ordinary absorptiometric methods, computer programs are needed to obtain reliable analytical results in shorter intervals.

The determination of food additives using conventional methods is difficult because of high-cost instruments and time-consuming pretreatment technique separations, such as extraction liquid–liquid, chromatography in column or fine plate [1]. Besides, equipment such as liquid and gas chromatography [1,2] are not available for small laboratories due to their high cost. This is the case for the determination of the benzoic and sorbic acid in food samples, which are employed as antimicrobial species in a wide number of foods, fruit juices, jams, beverages, salads, etc. [3].

On the other hand, there are spectrophotometric methods which require low-cost equipment and they can incorporate powerful chemometric tools of data analysis, such as the partial least square regression (PLS) multivariate calibration method. This low-cost analytical system avoids the time-consuming process during previous separation techniques, which may incorporate contamination.

In recent works, benzoic and sorbic acids in fruit juices were studied by Marsili et al. [4,5], using other multivariate calibration methods such as net analyte signal [4] and second-order spectrophotometric data [5]; yet, the PLS-2 multivariate calibration method has not been used, so far, to evaluate these analytes. For this reason, this paper discusses the simultaneous determination of benzoic and sorbic acids present in commercial samples of fruit juices, by means of the

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PLS-2 multivariate calibration method validated by the HPLC method.

1.1. The PLS method

Partial least square (PLS) regression is an important multivariate calibration tool based on the use of a large number of variables, which permits to evaluate the concentration of interesting analytes [6,7]. PLS can be used in two ways: PLS-1 calculates the concentration of one analyte per model, while PLS-2 can determine all analytes in a unique model. The PLS method is an important multivariate calibration tool that has been growing in importance for the last years and has been incorporated in new analytical chemistry textbooks [8].

PLS regression [9] is a full-spectrum method based on the resolution of two initial multivariate matrices, R (response matrix) and C (concentration matrix), by projection onto smaller matrices T and U (or R and C score matrices, respectively). They contain the coordinates of the objects on the new axes or PLS components, with orthogonal columns, and relates the information in the response matrix R to the concentration matrix C, through correlation between R and C covariance matrices. In this work, R represents the independent variables (the original absorbance data of the calibration set), while C represents the dependent variables (concentration of benzoic and sorbic acids in the calibration set). The determination of a significant number of model dimensions (number of PLS principal components) was made by cross-validation.

The PLS-2 method was employed using absorbance values every 2 nm, from 210 to 300 nm wavelength. Forty standard solutions were prepared to generate the response matrix R, adding known concentration of benzoic and sorbic acids and constant quantities of natural juices, to simulate the matrix effect during the calibration step.

In this work, five samples of commercial fruit juices were analyzed and the results were validated using high performance liquid chromatography (HPLC) [10,11].

2. Experimental

2.1. Reagents

Water: HPLC-grade water was used to prepare both standard and sample solutions. Sodium benzoate and potassium sorbate stock solutions (10 g L^{-1}), 1.000 g of sodium benzoate and 1.000 g of potassium sorbate ACS grade (Baker, Phillipsburg, NJ, USA) were diluted with HPLC-grade water into a 100 mL volumetric flask. For the PLS-2 method, all standard solutions (calibration and validation sets) were prepared diluting adequate volumes of stock solutions with HCl 5×10^{-4} mol L^{-1} into 100 mL volumetric flasks, to obtain pH values around 3 [4]. For the HPLC method, standards were prepared diluting adequate volumes of stock solutions with mobile phase into 100 mL volumetric flasks. Mobile phase: 20% acetonitrile HPLC grade (Merck, Durmsted, Germany) and 80% sodium acetate–acetic acid buffer solution prepared with HPLC-grade water.

Sample preparation. For the PLS-2 method, five commercial juices were prepared transferring 1 mL of each juice into a 100 mL volumetric flask diluted with HCl 5×10^{-4} mol L^{-1}. For the HPLC method, 1 mL of each filtered juice was transferred into a 100 mL volumetric flask and diluted with the HPLC mobile phase.

2.2. Instrumental

Spectrophotometric measurements were taken using a Metrolab 1700 UV-V spectrophotometer (Buenos Aires, Argentina), Czerny Turner monochromator and a photomultiplier detector. pH measurements were taken with a pH meter HORIBA F42 (Tokio, Japan). The HPLC data were obtained by KONIK KNK-500-A Series (Miami, FL, USA). A 25 cm C-18 column Lichrosorb RP18 (USA) was used with KONIK UV detector (Miami, FL, USA). The HPLC parameters were: flow rate 1 mL min^{-1}; injection 20 μL; wavelength 234 nm and a chromatographic time of 15 min per sample. The PLS-2 data analysis was carried out using the Unscrumbler 6.11 software (CAMO ASA, Trondheim, Norway).

3. Results and discussion

3.1. HPLC data

Six standard solutions and six replicates of each one were prepared for both benzoic and sorbic acids, with concentrations of 2.0, 4.0, 6.0, 10.0, 14.0 and 20.0 ($\times 10^{-3}$ g L^{-1}). HPLC conditions were similar to those stated by Pylypiw and Grether [10], but using a single wavelength at 234 nm. The obtained calibration curve yielded a r^2 regression coefficient of 0.9947 and 0.9972 for benzoic and sorbic acids, respectively.

3.2. The PLS model

The model was obtained using a total of 40 standard solutions of filtered natural apple, orange, lemon and grapefruit juices and adding different levels of sorbic and benzoic acids to each one: 0.0, 2.5, 5.0, 7.5 and 10.0 ($\times 10^{-3}$ g L^{-1}). As shown in a previous study [12] a factorial design was used to build the calibration matrix, with five levels and two variables. Nevertheless, another 15 standard solutions were prepared to obtain 10 standard solutions for each fruit juice. Spectrophotometrical readings were carried out in different days in order to bring more robustness to the PLS-2 model and to produce minor error levels in the prediction step. This is an important aspect due to the use of complete full-spectra data which are affected by the instrumental variations, producing little changes in absorbance values and significant levels of noise. The concentration matrix used in the calibration step is shown in Table 1. All standard solutions were read from 210 to 300 nm every 2 nm. Standard solutions 1–10 were prepared with lemon; 11–20 with orange; 21–30 with grapefruit and 31–40 with apple natural juices to obtain a unique model useful for all samples.

The PLS-2 model was made using the Unscrumbler 6.11 software tools. The calibration step was performed by the

Table 1
Concentration matrix for the PLS-2 model

Std	Benzoic acid[a]	Sorbic acid[a]	Std	Benzoic acid[a]	Sorbic acid[a]
1	0.0000	0.0100	21	0.0000	0.0100
2	0.0025	0.0075	22	0.0025	0.0075
3	0.0050	0.0050	23	0.0050	0.0050
4	0.0075	0.0025	24	0.0075	0.0025
5	0.0100	0.0000	25	0.0100	0.0000
6	0.0025	0.0100	26	0.0025	0.0100
7	0.0050	0.0075	27	0.0050	0.0075
8	0.0075	0.0050	28	0.0075	0.0050
9	0.0100	0.0025	29	0.0100	0.0025
10	0.0100	0.0100	30	0.0100	0.0100
11	0.0000	0.0100	31	0.0000	0.0100
12	0.0025	0.0075	32	0.0025	0.0075
13	0.0050	0.0050	33	0.0050	0.0050
14	0.0075	0.0025	34	0.0075	0.0025
15	0.0100	0.0000	35	0.0100	0.0000
16	0.0025	0.0100	36	0.0025	0.0100
17	0.0050	0.0075	37	0.0050	0.0075
18	0.0075	0.0050	38	0.0075	0.0050
19	0.0100	0.0025	39	0.0100	0.0025
20	0.0100	0.0100	40	0.0100	0.0100

[a] Concentrations expressed in g L^{-1}.

combination of the response matrix (R) 40 × 45: (40 standard solutions × 45 wavelength absorbance values) and the concentration matrix (C): (40 standard solutions × 2 analytes). The built model was obtained using auto scaled data.

Fig. 1 shows the spectral overlapping of benzoic and sorbic acids in a 210–300 nm range.

Table 2 shows the explained variance (cumulative percentage) obtained in the calibration and validation processes with the PLS-2 method. Four PLS components were needed to explain 99.6 and 99.7% of the original information for both, benzoic and sorbic acids in the calibration and validation model sets. The percentage of the explained variance, as well as the root mean square error of calibration (RMSEC) and the root mean square error of prediction (RMSEP) are important diagnostic tools [7]. The percentage of variance represents the amount of

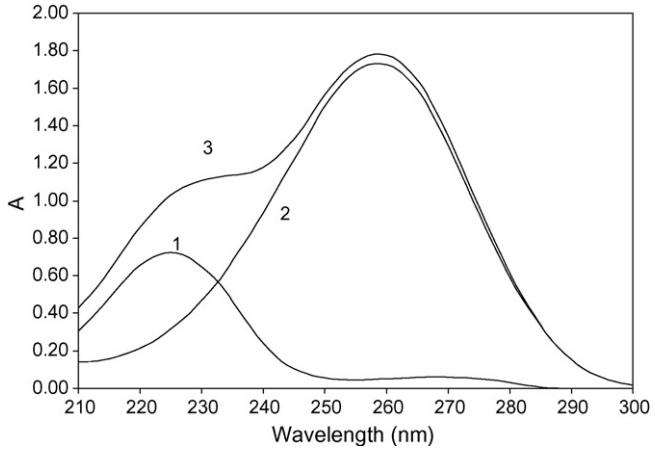

Fig. 1. Spectral curves of benzoic, sorbic and mixture of acids $C_{Benz} = C_{Sorb} = (1/2)C_{Mixt} = 0.01$ g L^{-1}.

Table 2
Percentage of explained variance for benzoic and sorbic acids in the calibration and validation model set

PC	Benzoic acid		Sorbic acid	
	Calibration	Validation	Calibration	Validation
0	0	0	0	0
1	33.2	27.2	95.2	95.2
2	58.2	54.1	97.6	97.5
3	85.4	82.7	97.5	97.2
4	99.6	99.6	99.8	99.7

variance explained by the PLS-2 model with a given number of PLS principal components, relative to the total variance in the R and C matrices in the data set. Another model evaluation tool is shown in Fig. 2, in the observed predicted concentration plot for benzoic (a) and sorbic (b) acids. The obtained r^2 coefficients were 0.9966 and 0.9979, respectively, suggesting a good fit in the model.

In Fig. 3, the loading weight plot, shows the PLS principal components' behavior as function of wavelength, describes the influence of wavelength for each principal component and represents their relative contribution to the model [7].

Frequently the loading plot is similar to the spectra of pure components. The first PLS principal component has a minimum at 262 nm, coinciding with the maximum of sorbic acid

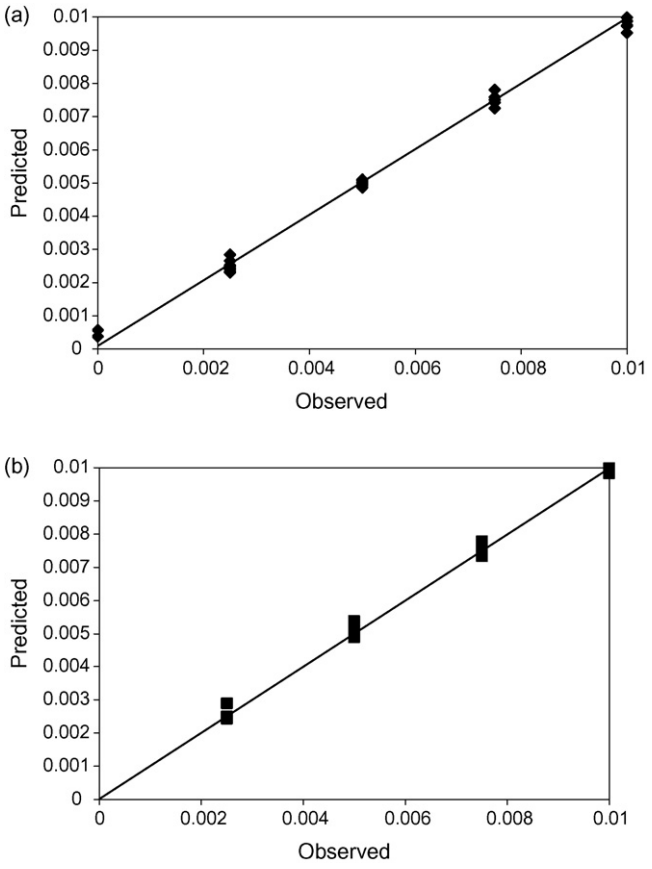

Fig. 2. Observed predicted plot, obtained by the cross-validation model with four principal components: (a) Benzoic (g L^{-1}) and (b) sorbic acids (g L^{-1}).

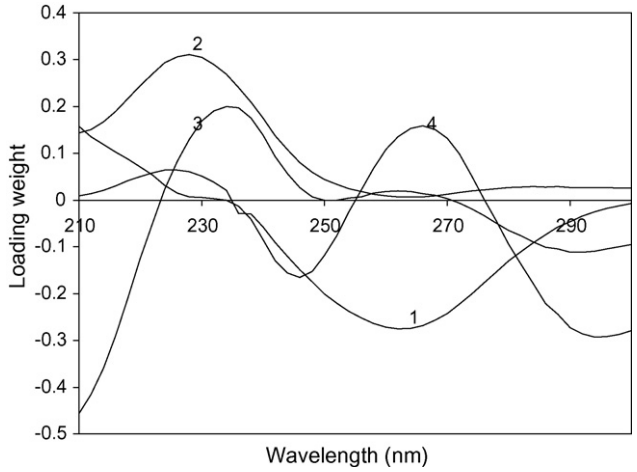

Fig. 3. Loading weights as function wavelength for first (1), second (2), third (3) and fourth (4) PLS principal components.

spectra, and a maximum that is in agreement with the maximum at 225 nm of benzoic acid spectra. The second component describes almost exactly the benzoic acid spectra. The third component shows an influence range from 210 to 250 nm with a maximum at 235 nm, which is the wavelength crossing of both pure spectra. The fourth component presents a minimum at 245 nm that corresponds to the inflection wavelength in the benzoic acid spectra; at 265 nm coincides with maximum of sorbic acid spectra, and at 295 nm agrees with the inflection zone of both benzoic and sorbic acid spectra.

3.3. Model validation

The model was built using, in the first place, internal validation (cross-validation method), where the model leaves out one standard of the calibration set. This standard was used to predict and find the internal error of the model. When all standards were left out once, the calibration and validation model error could be calculated, through root mean square of calibration (RMSEC) and root mean square of prediction (RMSEP) [6,7]. Fig. 4 shows RMSEC and RMSEP of the PLS-2 model for sorbic and benzoic acids.

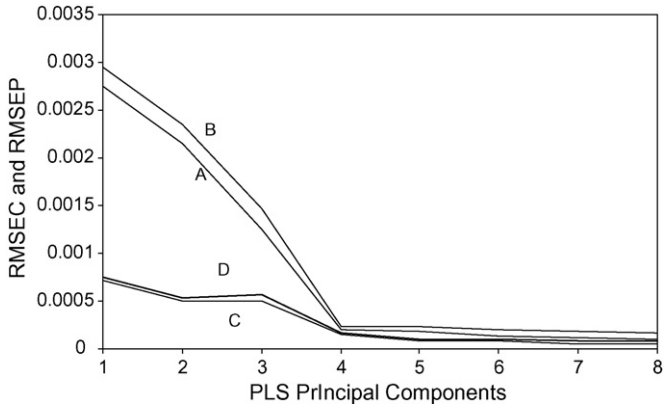

Fig. 4. RMSEC and RMSEP for benzoic and sorbic acids. Benzoic acid: (A) calibration, (B) validation. Sorbic acid: (C) calibration, (D) validation.

Table 3
Results of obtained prediction in the validation set for the PLS-2 model

Sample	Benzoic acid		Sorbic acid	
	Reference[a]	Predicted[a]	Reference[a]	Predicted[a]
Lemon 1	0.0030	0.0029	0.0060	0.0060
Lemon 2	0.0060	0.0062	0.0030	0.0029
Orange 1	0.0030	0.0029	0.0060	0.0063
Orange 2	0.0060	0.0056	0.0030	0.0033
Grapefruit 1	0.0030	0.0029	0.0060	0.0060
Grapefruit 2	0.0060	0.0061	0.0030	0.0029
Apple 1	0.0030	0.0031	0.0060	0.0059
Apple 2	0.0060	0.0061	0.0030	0.0027

[a] Concentrations expressed in g L^{-1}.

This figure also shows that four PLS principal components were properly chosen, because there were no significant changes in error values after the fourth component.

Then, a second validation was carried out through a validation set, preparing standard solutions of filtered natural juices, with concentration levels different from those used for the PLS-2 model to find the prediction error. The purpose of the validation set was to predict concentrations as unknown samples, to find errors and also the predictive ability of the model. Table 3 shows the results obtained with eight validation samples, which were prepared using lemon, orange, grapefruit and apple natural juices; benzoic and sorbic acids known concentrations were the references while the predicted values were the results. In this case, model relative error of less than 10% was found in all validation sample sets.

3.4. Outlier samples in the prediction step

Outlier detection during prediction in calibration methods is primarily based on *X*-residuals (response matrix residuals) and the prediction leverage. The *Y*-residuals (concentration matrix residuals) do not exist in the prediction step and consequently, measurements based on *f* cannot be used. *F* is the response error matrix obtained in the calibration step, and *f* (if it were possible to calculate it) must be the error matrix of an unknown sample in the prediction step. In the calibration step, detected outliers can be removed to obtain an adequate calibration model. In the prediction step, the leverage h_i summarizes extremeness in all factors applied in the modeling. Like the leverage of the calibration set, the leverage of the prediction set is defined as a truncated Mahalanobis distance. For outlier detection, the prediction leverage can be tested against the average leverage of the *I* object in the calibration set as follows:

$$h_i = \frac{k(A+1)}{I}$$

where *A* is the number of PLS-2 components and *k* is a constant, e.g. 3. In spite of the fact that leverage could never be higher than 1 in the calibration set, in the prediction step this limitation does not apply, and new input spectra can generate large factor scores.

In this work, the obtained leverage values for the real samples analyzed were the following: lemon (Leader Price) 0.092;

Table 4
Predicted concentrations by the PLS-2 model in real samples and validation results by the HPLC method

Sample	Benzoic acid		Sorbic acid	
	PLS-2[a]	HPLC[a]	PLS-2[a]	HPLC[a]
Lemon	1.100 ± 0.102	0.997 ± 0.011	0.340 ± 0.073	0.327 ± 0.008
Orange 1	0.980 ± 0.026	0.902 ± 0.019	0.190 ± 0.019	0.181 ± 0.004
Orange 2	0.900 ± 0.019	0.827 ± 0.019	0.000	0.000
Grapefruit	1.040 ± 0.049	0.971 ± 0.018	0.320 ± 0.035	0.310 ± 0.005
Apple	0.350 ± 0.010	0.335 ± 0.012	0.000	0.000

[a] Concentrations in undiluted bottled juices expressed in g L^{-1}.

orange 1 (Leader Price) 0.227; orange 2 (Zulueta) 0.178; grapefruit (MiJu) 0.228 and apple (Delifrú) 0.326. The low leverage values obtained show clearly that there are no sample outliers for this analyzed data set. Thus, this fact provides additional support for the very low influence of possible interferences on the analyzed samples [9].

3.5. Real samples prediction and HPLC validation results

Five samples with three replicates of commercial juices were predicted by the PLS-2 model: lemon (Leader Price), orange 1 (Leader Price), orange 2 (Zulueta), grapefruit (MiJu) and apple (Delifrú). Results were validated by the HPLC method.

The results obtained by the PLS-2 and HPLC methods are shown in Table 4. The relative error between the PLS-2 and HPLC methods was less than 12% in all cases.

On the other hand, the chromatogram shows that there are not relevant species that absorb at 234 nm (almost in the center between 210 and 300 nm range) in a large chromatographic time (60 min), which allows us to suppose that interfering absorbance values are not significant with respect to benzoic and sorbic acid values, due to the dilution phenomena, as can be seen through the low relative error and low leverage values for the sample set.

To summarize, the results obtained point out that the spectrophotometric methods combined with the PLS-2 data analysis permit the simultaneous determination of benzoic and sorbic acids in commercial fruit juices. The proposed method can be used without previous chemical separations, which suggests the great potential of the PLS-2 method with non-expensive equipment. Besides, it offers fast and precise results becoming an alternative procedure for laboratories of routine analysis.

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