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NON-DESTRUCTIVE FLUORESCENCE SPECTROSCOPY COMBINED WITH SECOND-ORDER CALIBRATION AS A NEW STRATEGY FOR THE ANALYSIS OF THE ILLEGAL SUDAN I DYE IN PAPRIKA POWDER

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Abstract

This paper presents a novel strategy for determination of the illegal dye Sudan I in paprika powder. The method is based on fluorescence spectroscopy combined with second-order calibration, which was employed for the first time for this purpose. The method is non-destructive and requires no sample preparation. It was probed that Sudan I exhibited fluorescence; however, the color of paprika samples affected the signal and it was not possible to quantify this adulterant by means of univariate and first-order calibration. To model the effect of variability of color in samples, a central composite experimental design was performed with varying ASTA (American Spices Trade Association) color values and Sudan I concentrations. Different second-order algorithms were tried for quantification. The best results for calibration and validation were obtained from Unfolded-Partial Least-Squares (U-PLS) and Multi-way Partial Least-Squares (N-PLS). The level of detection ranges were 0.4 - 3 mg/g and 0.5 - 3 mg/g for U-PLS and N-PLS, respectively. This is lower than other methods found in the literature.

Keywords: paprika powder, fluorescence spectroscopy, non-destructive analysis, second-order calibration, Sudan I

1. Introduction

Sudan dyes are classified as a family of azo dyes used in industrial and scientific applications. These compounds are considered attractive due to their low-cost and widely availability and could be used in food as colorants [1]. However, these compounds present toxic properties and their use in food is prohibited. Sudan I [1-(phenylazo)-2-napthtol] belongs to this family and its structure is shown in Figure 1. Sudan I is among the most common compounds employed for adulteration of foods, such as chili (sauce and powder), paprika powder, tomato sauces, etc. [2].

Paprika powder is obtained from milled peppers. This product is being increasingly consumed as spice in cookery. The color of paprika powder is one of the most important quality parameters, which could be affected by storage-time. Furthermore, the production conditions may affect the color of paprika powder. Therefore, adding illegal colorants could be tempting [3] to increase value and reduce production costs.

There are many methods present in the literature where Sudan I has been quantified and/or detected in paprika, peppers or similar matrices. A deep review of the methods developed in the last decade shows that the analytical techniques employed have been very diverse, from spectrophotometry to electrochemical techniques (Table 1).

Separation techniques have been employed for quantification purposes. A review from 2010 shows a number of methods employing liquid chromatography with different detection modes [1]. All methods require sample treatment by extraction of Sudan dyes with solvents. The majority of the studies used a conventional C18 column. Low detection limits were obtained in these studies. After 2010, other methods have been published using liquid chromatography with UV detection for determination of different Sudan dyes (Sudan I - IV) and different samples (tomato sauce, chili powder, candies or water) [4–7]. These techniques require more instrumentation set-up and treatment of samples as compared to e.g. spectroscopic or electrochemical techniques.

Other common techniques used for determination of Sudan compounds are electrochemical techniques, using modified electrodes, like cyclic voltammetry (CV) [8,9], square-wave voltammetry (SWV) [9] and differential pulse voltammetry (DPV) [10,11]. These studies [9–11] quantified Sudan I in extracts from food matrices. Moreover, Heydari et al. [11] resolved a mixture of dyes (Sudan II and III) by using the chemometric algorithm multivariate curve resolution-alternating least-squares (MCR-ALS) with the corresponding voltammograms from samples.

Also, UV-Vis spectrophotometry has been used in the determination of Sudan dyes. Some of these studies have been performed for classification purposes (Sudan dyes present or not) [13,15–17], others for quantification purposes [12] and, in some cases, both [14]. Partial Least-Squares discriminant-analysis (PLS-DA) was used in these studies for classification and Partial Least-Squares Regression (PLSR) for quantification. Furthermore, Parallel Factor Analysis (PARAFAC) was applied in one study where they employed second-order data for determination of Sudan I in chili powder, obtained from solvent components gradual change-visible spectra [12]. With the aforementioned methods the dyes were determined in different foods (chili, turmeric, curry, paprika, sauces, etc.) but all the methods required an extraction step before determination.

Haughey et al. quantified Sudan I in chili, without any sample pre-treatment, employing Fourier Transform Raman spectroscopy [18]. In a recent work, we used dispersion Raman spectroscopy with 785 nm excitation laser to quantify Sudan I in intact paprika samples [3], removing fluorescence background by mathematical pre-treatment of the spectra [19]. Surface enhanced Raman spectroscopy has also been used for classification of samples based on the content of different dyes (Sudan I, Rhodamine-b and malachite green) [20] and for quantification of Sudan III in paprika powder [21]. Recently, Deng et al. [2] employed this technique for quantifying Sudan I in chili and tomato sauce with low detection and quantification limits. Also, when SERS is used, the extraction of targeted dyes from samples was required.

Fluorescence spectroscopy is a potential technique in the analysis of foods. However, in the case of Sudan compounds, it has not been extensively tested. In the literature, there are a few works were this technique was employed. Di Anibal et al. used synchronous fluorescence with multivariate classification techniques to detect Sudan I in extracts of samples from different paprika varieties [22]. In a recent study, Anmei et al. have quantified Sudan I in different foods based on the quenching effect that this compound presented in the fluorescence spectra of carbon quantum dots prepared from cigarette filters. The decrease in signal intensity was related to Sudan I concentration [23] in the ethanol solvent extracts.

Moreover, fluorescence has been used in the development of different sensors or assays. Huang et al. reported a fluorescence assay for Sudan I and Sudan III based on the ligand exchange of Cu (II) - calcein complex when Sudan I or III are present in the media [24]. Another nanosensor for sensitive fluorescence detection of Sudan I-IV has been developed by Fang et al. [25]. In this case, the detection was based on fluorescence quenching of hexadecyl trimethyl ammonium bromide stabilized upconversion nanoparticles through the inner filter effect. In presence of Sudan dyes, the nanoparticles fluorescence emission decreased due to the absorption bands of Sudan dyes. With this sensor, Sudan I-IV in chili powders were tested with a standard addition method, showing good selectivity, sensitivity and successfully application to detect Sudan in chili powder samples.

Note that when a treatment of samples is required, methods are expensive with respect to time and solvents. For this reason, developing rapid, affordable and environmentally friendly methods is important.

For this purpose, the autofluorescence measurements combined with chemometrics is a potential tool. A recent review [26] shows that most studies obtaining autofluorescence measurements on food matrices were applied to liquid samples. Also, this technique offered promising results for meat [27], fish [27], cocoa [28] or dairy products [29], among others. From our knowledge, in the case of paprika powder, no study has been performed until now.

Given the selectivity and sensitivity offered by fluorescence spectroscopy, the main objective of this work was to explore the possibility of employing non-destructive fluorescence in the analysis of the illegal dye Sudan I in paprika powder.

2. Experimental

2.1. Chemical and samples

Sudan I (≥ 95 %) was purchased from Sigma-Aldrich (St. Louis, MO). The different paprika powder samples, that were used in the study, were obtained from the Spanish Protected Designation of Origin (PDO) *"Pimentón de La Vera"* (n = 6) and from Spanish (n = 1) and Norwegian (n = 3) local markets.

They had a wide variability in color, defined by the ASTA value. The ASTA color value is a scale of the American Spices Trade Association (ASTA), which determines if the paprika is of high quality or not based on its ASTA value. Also, some PDO presents a threshold for considering a paprika powder sample belonging or not to this PDO. The ASTA color value and the origin for each sample are shown in the Table 2. Onwards these samples IDs are used in all the tables and figures.

2.2. Calibration and validation sets description

In this study two calibration sets and one validation set were used. The calibration set 1 was made based on one paprika sample with a high ASTA value (ASTA = 149). Aliquots of this sample were adulterated with different amounts of Sudan I standard, resulting in one pure sample and six adulterated samples (Table 3). For the calibration set 2, The Unscrambler® (version 9.7, CAMO Software 2007) was used to obtain the experimental design (Central Composite Experimental Design). The two parameters varied were ASTA color values and Sudan I concentration. ASTA color values of paprika powder varied between 25 and 150 based on selected paprika samples and the samples were spiked with Sudan I dye at several concentrations, between 0.27 and 24 mg/g.

This design resulted in a total of 9 samples (Table 3) with different composition (5 different levels). Concentrations shown in the table are the final concentration after accurate weighing of samples. Additionally, the five pure paprika samples employed in the experimental design were also included in the calibration set, resulting 14 samples for the calibration set. The validation set was formed by 9 samples with different ASTA values and Sudan I concentration (Table 3).

The calibration set 1 and the validation set were used for univariate calibration, first- and second-order calibrations in the first step of this study. The calibration set 2 and the validation set were used for first- and second-order calibrations in the second part of this study.

In order to obtain the spiked adulterated samples, exact amounts of paprika and Sudan I standard were weighted and manually mixed until homogenous.

2.3. Excitation - emission matrices (EEMs) acquisition

A Fluoromax-4 spectrofluorometer (Horiba Scientific), equipped with two Czerny-Turner monochromators, a xenon lamp and a photomultiplier tube as detector, was employed to collect the excitation - emission matrix of each sample. Measurements were performed with a fiber optic probe (J1950 fiber-optic bundles) plus FM-4-300 fiber optic mount couple to the sample compartment, and without direct contact with samples. Analysis was non-invasive and non-destructive. The emission spectra were collected from 420 to 800 nm, each 3 nm, varying the excitation wavelength from 400 to 500 nm, in 5 nm steps. Excitation and emission slits widths: 5 nm. Each sample was measured in triplicate.

2.4. Data analysis

PLSR was employed for the analysis of first-order signals. In the case of second-order data, Parallel Factor Analysis (PARAFAC), Unfolded-Partial Least-Squares (U-PLS) and Multi-way Partial Least-Squares (N-PLS) were applied and compared. All data analysis was done in Matlab

® R2007b (version 7.5.0.342) with the mvc1 and mvc2 routines developed by Olivieri et al. [30,31] and available at [32,33].

Limits of detection (LODs) were calculated as model performance parameters. Currently, there is no well-defined procedure for providing LODs in multivariate calibration. Some studies suggest to use a LOD interval [34,35]. These LOD intervals were calculated, using the mvc2 routine, according to:

$$LOD_{min} = 3.3[SEN^{-2}var(x) + h_{0min}\, SEN^{-2}var(x) + h_{0min}var(y_{cal})]^{1/2} \quad (1)$$

$$LOD_{max} = 3.3[SEN^{-2}var(x) + h_{0max}\, SEN^{-2}var(x) + h_{0max}var(y_{cal})]^{1/2} \quad (2)$$

Where SEN is sensitivity, *var(x)* is the variance in the instrumental signals, *var(y_{cal})* is the variance in the calibration concentrations, h is the sample leverage, being h_{0min} and h_{0max}, the minimum and maximum values of this parameter for a certain calibration set. More details can be found at [34,35].

3. Results and discussion

3.1. Sudan I fluorescence

The fluorescence of Sudan I was previously described by Di Anibal et al. [22]. They reported an emission maximum for excitation/emission wavelengths of 420/550 nm obtained from an isopropyl alcohol Sudan I extract. In our case, we found a maximum for the excitation/emission wavelengths of 465/588 nm (Figure 1) for pure Sudan I. The position of the maximum was shifted to longer wavelengths (30 - 40 nm) when spectra were obtained from intact solid samples rather from a solution. These changes could be attributed to the variation that molecules suffer in solution compared with solid samples, in the same way that their profiles might change with different solvents.

3.2. Univariate calibration

For univariate calibration, emission spectra for excitation at 465 nm from paprika, with different levels of Sudan I adulteration, were extracted from the EEMs of calibration set 1 of samples, shown in Figure 2a. With increasing concentration of Sudan I, one would expect a corresponding increase in fluorescence intensity. However, a non-linear relationship was observed (Figure 2b) when regression was obtained between fluorescence intensity at maximum for Sudan I and the concentration of Sudan I. Univariate calibration was, therefore, not appropriate for quantification. This could be due to inner filter effects or to the fact that other compounds, present in paprika samples, cause a matrix effect interfering in the determination of Sudan I by means of univariate analysis. For this reason, first- and second-order calibrations were investigated.

3.3. First- and second-order calibration

The first step in the multivariate analysis performed was to obtain a calibration model employing PLSR on calibration set 1. From the EEMs collected, emission, spectra were extracted from 480 to 800 nm for the excitation wavelength of 465 nm. A PLSR model, based on 3 components, explained 99.9 % of the variance, in Y, and the coefficient of determination (R^2) was 0.984 for the calibration model. However, when the validation set with different paprika samples were predicted by this model, high prediction errors were obtained (Table 4). These high errors could be related with the fact that validation samples had different ASTA values compared to those in the calibration set. This indicates that the color of paprika could have influence in the Sudan I fluorescence signal.

In order to explore whether second-order calibration offered better results, the algorithms U-PLS and N-PLS were tested on calibration set 1. In these cases, to avoid the Rayleigh dispersion in the EEMs, a selected region was employed in the analysis (excitation wavelength from 400 to 500 nm and emission wavelength from 531 to 630 nm). This region was employed in the further second-order analysis.

198 For U-PLS and N-PLS, the optimal number of components were selected using the Haaland and
199 Thomas criterion [36,37], and two components were obtained. Results for calibration and
200 validation results are shown in Table 4. Again, the relative error of predictions (REPs) in the test
201 samples were higher than 50 %, which confirms the fact that color of paprika could be influencing
202 in the Sudan I fluorescence signal and it should be modelled.

203 Figure 3 shows the EEMs for different paprika samples. First, it is seen that samples with low
204 ASTA values, exhibited higher fluorescence intensities around 465 and 550 nm for excitation and
205 emission, respectively. Also, it is observed that when the Sudan I concentration increases, this
206 signal decreases, which could be because Sudan I absorbs the excitation light. Finally, different
207 shapes were observed for Sudan I present in the same concentration in paprika samples with
208 different colors, probably due to the absorption of light from paprika carotenoids.

209 After this, calibration set 2 was obtained and samples were measured, containing variation also
210 in ASTA values. This calibration set has more variability than calibration set 1, which makes the
211 models more robust.

212 First and second-order calibration were performed with these data sets. Results obtained for the
213 different calibration models are shown in the Table 4. In the case of first-order calibration,
214 emission spectra from 480 to 800 nm were again selected at the maximum of excitation (465 nm).
215 PLSR was employed for building the calibration model. In this case, a two-component model was
216 selected, explaining 96.5 % of variation in Sudan I concentration. For this calibration model, the
217 R^2 was 0.956, which is acceptable. However, higher REP value was obtained than when the first
218 calibration set was used. Moreover, when the model was validated, RMESP was 5.1 mg/g and
219 REP was 45 %. These results showed that the errors were slight high for first-order calibration.

220 In the case of PARAFAC, a model based on 3 components was obtained taking into account
221 different criteria [38–41]. However, due to the lack of trilinearity in the data, even when the

222 variability of color was included in the calibration set 2, this algorithm failed in the calibration
223 and validation steps ($R^2 < 0.3$).

224 In the case of U-PLS and N-PLS, the optimal number of components was 5 for both methods
225 based on the Haaland and Thomas criterion [36,37]. Table 4 shows that the two methods
226 performed equally well. To validate the models, we used the validation set. Plotting known values
227 against predicted values of Sudan I concentration for the validation samples gave good results
228 and lower RMSEP than with first-order calibration (Table 4).

229 The results for our study suggest that, U-PLS and N-PLS were more robust algorithms that can
230 take into account trilinearity deviations caused by matrix effects, inner filter effects and strongly
231 overlapping of spectra. U-PLS and N-PLS can model the lack of trilinearity including the
232 variability of samples in the calibration set, for example, including a pool of sample background
233 in the calibration set in case of matrix effect [42,43].

234 Moreover, if some uncalibrated interferents would be present in further samples, U-PLS and N-
235 PLS could be coupled to residual bilinearization (RBL) approach for solving and modelling the
236 interferents.

237 In the case of U-PLS and N-PLS, the LODs were calculated as detailed in the section 2.4. Hence,
238 the LODs were in the range of 0.4 - 3 mg/g and, 0.5 - 3 mg/g, for U-PLS and N-PLS, respectively.
239 These limits can be compared with others obtained with spectroscopic techniques. For instance,
240 the study of Márquez et al. based on the analysis of Sudan I by UV spectroscopy, with a previous
241 extraction of dyes, provided a LOD for Sudan I of 1.5 mg/g [15]. Also, a recent work developed
242 in our group [3] offered a detection capability of 5 mg/g.

243 Concentrations of Sudan I in 100 - 1000 mg/kg range are required to impact the color of chili
244 products [1]. For this reason, this method could be a good alternative to use as screening in case
245 that Sudan I is added to improve color of paprika powder.

4. Conclusions

This study shows that autofluorescence can be applied directly on paprika powder for the determination of Sudan I concentration. Furthermore, the lack of trilinearity, due to the variability of color in samples, could be handled by including this variation as part of the calibration. U-PLS and N-PLS algorithms have been proved better for solving the lack of trinilinearity that PARAFAC.

This method is quick, non-destructive and easy to use, being a good alternative to other methods. However, more samples should be included in further studies to prove if this method can be also used as classification method of adulterated or not adulterated samples. Furthermore, it would be interesting study the possibility of quantifying several Sudan dyes at the same time.

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References

[1] R. Rebane, I. Leito, S. Yurchenko, K. Herodes, A review of analytical techniques for determination of Sudan I-IV dyes in food matrixes, J. Chromatogr. A. 1217 (2010) 2747–2757.

[2] D. Deng, H. Yang, C. Liu, K. Zhao, J. Li, A. Deng, Ultrasensitive detection of Sudan I in food samples by a quantitative immunochromatographic assay, Food Chem. 277 (2019) 595–603.

[3] O. Monago-Maraña, C.E. Eskildsen, N.K. Afseth, T. Galeano-Díaz, A. Muñoz de la Peña, J.P. Wold, Non-destructive Raman spectroscopy as a tool for measuring ASTA color values and Sudan I content in paprika powder, Food Chem. 274 (2019) 187–193.

[4] Y. Li, Y. Wang, H. Yang, Y. Gao, H. Zhao, A. Deng, Establishment of an immunoaffinity chromatography for simultaneously selective extraction of Sudan I, II, III and IV from food samples, J. Chromatogr. A. 1217 (2010) 7840–7847.

[5] M. Rajabi, S. Sabzalian, B. Barfi, S. Arghavani-Beydokhti, A. Asghari, In-line micro-matrix solid-phase dispersion extraction for simultaneous separation and extraction of Sudan dyes in different spices, J. Chromatogr. A. 1425 (2015) 42–50.

[6] W. Yu, Z. Liu, Q. Li, H. Zhang, Y. Yu, Determination of Sudan I-IV in candy using ionic liquid/anionic surfactant aqueous two-phase extraction coupled with high-performance liquid chromatography, Food Chem. 173 (2015) 815–820.

[7] M. Bazregar, M. Rajabi, Y. Yamini, S. Arghavani-Beydokhti, A. Asghari, Centrifugeless dispersive liquid-liquid microextraction based on salting-out phenomenon followed by high performance liquid chromatography for determination of Sudan dyes in different species, Food Chem. 244 (2018) 1–6.

[8] E. Prabakaran, K. Pandian, Amperometric detection of Sudan i in red chili powder samples using Ag nanoparticles decorated graphene oxide modified glassy carbon electrode, Food Chem. 166 (2015) 198–205.

[9] D. Thomas, A.E. Vikraman, T. Jos, K.G. Kumar, Kinetic approach in the development of a gold nanoparticle based voltammetric sensor for Sudan I, LWT - Food Sci. Technol. 63 (2015) 1294–1300.

[10] S. Palanisamy, K. Thangavelu, S.-M. Chen, V. Velusamy, S.K. Ramaraj, Voltammetric determination of Sudan I in food samples based on platinum nanoparticles decorated on graphene-β-cyclodextrin modified electrode, J. Electroanal. Chem. 794 (2017) 64–70.

[11] M. Heydari, S.M. Ghoreishi, A. Khoobi, Chemometrics-assisted determination of Sudan

dyes using zinc oxide nanoparticle-based electrochemical sensor, Food Chem. 283 (2019) 68–72.

[12] J. Yuan, L. Liao, Y. Lin, C. Deng, B. He, Determination of Sudan I in chilli powder from solvent components gradual change-visible spectra data using second order calibration algorithms, Anal. Chim. Acta. 607 (2008) 160–167.

[13] C. V. Di Anibal, S. Rodrí-guez, L. Albertengo, M.S. Rodrí-guez, UV-Visible spectroscopy and multivariate classification as a screening tool for determining the adulteration of sauces, Food Anal. Methods. 9 (2016) 3117–3124.

[14] C. Márquez, I. Ruisánchez, M.P. Callao, Qualitative and quantitative multivariate strategies for determining paprika adulteration with SUDAN I and II dyes, Microchem. J. 145 (2019) 686–692.

[15] C. V. Di Anibal, M. Odena, I. Ruisánchez, M.P. Callao, Determining the adulteration of spices with Sudan I-II-II-IV dyes by UV-visible spectroscopy and multivariate classification techniques, Talanta. 79 (2009) 887–892.

[16] C. V. Di Anibal, I. Ruisánchez, M. Fernández, R. Forteza, V. Cerdà, M. Pilar Callao, Standardization of UV-visible data in a food adulteration classification problem, Food Chem. 134 (2012) 2326–2331.

[17] D.N. Vera, I. Ruisánchez, M.P. Callao, Establishing time stability for multivariate qualitative methods. Case study: Sudan I and IV adulteration in food spices, Food Control. 92 (2018) 341–347.

[18] S.A. Haughey, P. Galvin-King, Y.C. Ho, S.E.J. Bell, C.T. Elliott, The feasibility of using near infrared and Raman spectroscopic techniques to detect fraudulent adulteration of chili powders with Sudan dye, Food Control. 48 (2015) 75–83.

[19] C.A. Lieber, A. Mahadevan-Jansen, Automated method for subtraction of fluorescence from biological Raman spectra, Appl. Spectrosc. 57 (2003) 1363–1367.

[20] S. He, W. Xie, W. Zhang, L. Zhang, Y. Wang, X. Liu, Y. Liu, C. Du, Multivariate qualitative analysis of banned additives in food safety using surface enhanced Raman scattering spectroscopy, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 137 (2015) 1092–1099.

[21] M. Jahn, S. Patze, T. Bocklitz, K. Weber, D. Cialla-May, J. Popp, Towards SERS based applications in food analytics: Lipophilic sensor layers for the detection of Sudan III in food matrices, Anal. Chim. Acta. 860 (2015) 43–50.

[22] C. V. Di Anibal, M.S. Rodríguez, L. Albertengo, Synchronous fluorescence and multivariate classification analysis as a screening tool for determining Sudan I dye in

329 culinary spices, Food Control. 56 (2015) 18–23.
330 [23] S. Anmei, Z. Qingmei, C. Yuye, W. Yilin, Preparation of carbon quantum dots from
331 cigarette filters and its application for fluorescence detection of Sudan I, Anal. Chim.
332 Acta. 1023 (2018) 115–120.
333 [24] S.T. Huang, L.F. Yang, N.B. Li, H.Q. Luo, An ultrasensitive and selective fluorescence
334 assay for Sudan I and III against the influence of Sudan II and IV, Biosens. Bioelectron.
335 42 (2013) 136–140.
336 [25] A. Fang, Q. Long, Q. Wu, H. Li, Y. Zhang, S. Yao, Upconversion nanosensor for
337 sensitive fluorescence detection of Sudan I-IV based on inner filter effect, Talanta. 148
338 (2016) 129–134.
339 [26] E. Sikorska, I. Khmelinskii, M. Sikorski, Fluorescence spectroscopy and imaging
340 instruments for food quality evaluation, in: J. Zhong, X. Wang (Eds.), Eval. Technol.
341 Food Qual., Elsevier Inc., 2019: pp. 491–533.
342 [27] A. Hassoun, A. Sahar, L. Lakhal, A. Aït-Kaddour, Fluorescence spectroscopy as a rapid
343 and non-destructive method for monitoring quality and authenticity of fish and meat
344 products: Impact of different preservation conditions, Lwt. 103 (2019) 279–292.
345 [28] J. Tan, R. Li, Z.T. Jiang, S.H. Tang, Y. Wang, Rapid and non-destructive prediction of
346 methylxanthine and cocoa solid contents in dark chocolate by synchronous front-face
347 fluorescence spectroscopy and PLSR, J. Food Compos. Anal. 77 (2019) 20–27.
348 [29] S. Shaikh, C. O'Donnell, Applications of fluorescence spectroscopy in dairy processing:
349 a review, Curr. Opin. Food Sci. 17 (2017) 16–24. doi:10.1016/j.cofs.2017.08.004.
350 [30] A.C. Olivieri, H.L. Wu, R.Q. Yu, MVC2: A MATLAB graphical interface toolbox for
351 second-order multivariate calibration, Chemom. Intell. Lab. Syst. 96 (2009) 246–251.
352 [31] A.C. Olivieri, H.C. Goicoechea, F.A. Iñón, MVC1: An integrated MatLab toolbox for
353 first-order multivariate calibration, Chemom. Intell. Lab. Syst. 73 (2004) 189–197.
354 [32] Instituto de Química de Rosario, MVC1: programa para calibración multivariada de
355 primer orden en MATLAB, (n.d.). www.iquir-conicet.gov.ar/descargas/mvc1.rar.
356 [33] Instituto de Química de Rosario, MVC2: programa para calibración multivariada de
357 segundo orden en MATLAB, (n.d.). www.iquir-conicet.gov.ar/descargas/mvc2.rar.
358 [34] F. Allegrini, A.C. Olivieri, IUPAC-consistent approach to the limit of detection in partial
359 least-squares calibration, Anal. Chem. 86 (2014) 7858–7866.
360 [35] A. Muñoz de la Peña, A.C. Olivieri, G.M. Escandar, H.C. Goicoechea, eds.,
361 Fundamentals and analytical applications of multiway calibration, in: Fundam. Anal.

Appl. Multiw. Calibration, Elsevier, Amsterdam, 2015: pp. 247–292.

[36] D.M. Haaland, E. V. Thomas, Partial Least-Squares methods for spectral analyses. 1. Relation to other quantitative calibration Methods and the Extraction of Qualitative Information, Anal. Chem. 60 (1988) 1193–1202.

[37] D.M. Haaland, E. V. Thomas, Partial Least-Squares methods for spectral analyses. 2. Application to simulated and gas spectral data, Anal. Chem. 60 (1988) 1202–1208.

[38] R. Bro, PARAFAC. Tutorial and applications, Chemom. Intell. Lab. Syst. 38 (1997) 149–171.

[39] A.C. Olivieri, G.M. Escandar, Practical Three-Way Calibration, Elsevier, Waltham, 2014.

[40] G.M. Escandar, H.C. Goicoechea, A. Muñoz de la Peña, A.C. Olivieri, Second- and higher-order data generation and calibration: A tutorial, Anal. Chim. Acta. 806 (2014) 8–26.

[41] A. Muñoz de la Peña, A. Espinosa Mansilla, D. González Gómez, A.C. Olivieri, H.C. Goicoechea, Interference-free analysis using three-way fluorescence data and the parallel factor model. Determination of fluoroquinolone antibiotics in human serum, Anal. Chem. 75 (2003) 2640–2646.

[42] D. Bohoyo Gil, A. Muñoz de la Peña, J.A. Arancibia, G.M. Escandar, A.C. Olivieri, Second-order advantage achieved by unfolded-partial least-squares/residual bilinearization modeling of excitation-emission fluorescence data presenting inner filter effects, Anal. Chem. 78 (2006) 8051–8058.

[43] M.V. Navarro, M.A. Cabezón, P.C. Damiani, Simultaneous determination of pesticides in fruits by using second-order fluorescence data resolved by unfolded partial least-squares coupled to residual bilinearization, J. Chem. 2018 (2018) 1–17.

[44] M. Gómez, V. Arancibia, M. Aliaga, C. Núñez, C. Rojas-Romo, Determination of Sudan I in drinks containing Sunset yellow by adsorptive stripping voltammetry, Food Chem. 212 (2016) 807–813.

[45] A.N. Berlina, A. V. Zherdev, C. Xu, S.A. Eremin, B.B. Dzantiev, Development of lateral flow immunoassay for rapid control and quantification of the presence of the colorant Sudan I in spices and seafood, Food Control. 73 (2017) 247–253.

Table 1. Analytical methods for the analysis of Sudan dyes in different foods.

Analyte	Matrix	Sample treatment	Analytical techniques	Chemometric algorithms	Classification/ Quantification	Other details	Ref.
Sudan I - IV	Tomato sauce, chili powder and chilli sauce	Extraction with immunoaffinity chromatography (IAC) columns	HPLC-UV	-	Quantification	After 50 times repeated usage of IAC columns, 64 % of the maximum capacity was still remained	[4]
Sudan I - IV	Chili	MMSPE with the following conditions: amount of sample, 0.0426 g; amount of dispersant phase, 0.0216 g of florisil, 0.0227 g of silica, 0.0141 g of alumina; and blending time, 112 s	HPLC-UV	-	Quantification	Low LODs and LOQs	[5]
Sudan I - IV	Candy	2.0 g of Candy simple was diluted with 4.00 mL water and placed into 10 mL centrifuge tube. 400 uL of C4[MIM]BF4 and 0.15 g of SDBS were added into the tube. The mixture was ultrasonically shaken for 3 min	HPLC -UV	-	Quantification	Low LODs for the four analytes	[6]
Sudan I - IV, Sudan orange G and Sudan red G	Turmeric, chili sauce and river and waste water	Centrifuge less DLLME. Extracting solvent: 1-undecanol was added to 10 mL of each sample solution repeating 13 times. Resulting mixture was passed at a flow rate of 2.0 mL min^{-1} through a small column filled with 5 g of sodium chloride, used as separation reagent. Extractant phase was solidified and collected for injecting in the HPLC system.	HPLC - UV	-	Quantification	Overall extraction time of 7 min	[7]
Sudan I	Chili, ketchup	2.0 g of simple was extracted with ethanol for 20 min.	CV, SWV	-	Quantification	Gold nanoparticle modified glassy carbon electrode (AuNO/GCE) was used as the working electrode and platinum wire as auxiliary electrode	[9]
Sudan I	Orange energy drinks	Solutions were prepared in phosphate buffer (pH 12.7)	AdSV	-	Quantification	Hanging mercury drop electrode (HMDE) as working electrode	[44]
Sudan I			DPV	-	Quantification	Platinum nanoparticles (PtNPs) decorated graphene/β-cyclodextrine (graphene/β-CD) modified electrode	[10]

Table 1. Analytical methods for the analysis of Sudan dyes in different foods.

Analyte	Matrix	Sample treatment	Analytical techniques	Chemometric algorithms	Classification/ Quantification	Other details	Ref.
Sudan I	Chili powder	-	CV	-	Quantification	Silver nanoparticles decorated graphene oxide modified glassy carbon electrode was used as working electrode. Amperometric detection	[8]
Sudan II and III	Chili and ketchup	1.0 g of chilli or ketchup sauce was weighed and added to 25.0 mL ethanol and ultrasonicated for 30 min	DPV	MCR-ALS	Quantification	Second-order data were obtained changing one instrumental parameter (pulse height). A surface of zinc oxide nanoparticles (ZnONPs) modified carbon paste electrode was used as working electrode	[11]
Sudan I	Chili powder	1 g of sample was weighted and 10 mL of ethanol was added. After 20 min, residue was evaporated and redissolved with cyclohexane	Spectrophotometry	RAFA, PARAFAC	Quantification	Second-order data were obtained adding different ethanol volumes to the cyclohexane extract from chilli	[12]
Sudan I, II, III and IV	Turmeric, curry and paprika	1 g of samples was extracted with acetonitrile and spiked with Sudan dyes	Spectrophotometry	KNN, SIMCA, PLS-DA	Classification	Spiked Sudan dyes samples up to 5 mg · L^{-1}	[15]
Sudan I - IV	Turmeric, curry and paprika	1 g of samples was extracted with acetonitrile and spiked with Sudan dyes	Spectrophotometry	PLS-DA	Classification	Piecewise direct standardization (PDS) was used to establish the relationship between the spectra of a sample measured under two different experimental conditions	[16]
Sudan I and III	Chili	300 □L of borate buffer solution, 100 □L of calcein, 50 □L of $CuSO_4$ and 50 □L of working solution (in ethanol) containing different concentrations of Sudan was added into 2 mL Eppendorf tube and diluted to 1 mL with water, and then the mixture was mixed	Fluorescence	-	Quantification	Sensor based on calcein liberation from the ligand exchange reaction in presence of Sudan I or III	[24]
Sudan I	Paprika	200 mg of paprika was extracted with isopropyl alcohol	Synchronous fluorescence	PLS-DA	Classification	First-derivative spectra improved classification results	[22]
Sudan I, Rhodamine B and Malachite green	Banned food additives	10 □L of sample was deposited on a gold-plated silicon SERS substrate and dried to get rid of solvent completely	SERS	PCA, PLS-DA	Classification	ICSF baseline correction was performed	[20]

Table 1. Analytical methods for the analysis of Sudan dyes in different foods.

Analyte	Matrix	Sample treatment	Analytical techniques	Chemometric algorithms	Classification/ Quantification	Other details	Ref.
Sudan I	Chili powder	None	NIR and Raman spectroscopy	PCA PLS-DA	Classification and quantification	LOD of 0.25 % for NIR and 0.88 % for Raman	[18]
Sudan III	Paprika	Extraction with methanol	SERS	-	Quantification	Employing of SERS active silver nanostructures. Formation of hydrophobic surface. Detection of Sudan III in presence of riboflavin as water-soluble competitor.	[21]
Sudan I	Sauces (ketchups and barbecue sauces)	10 mL of NN-dimethylacetamide was added to 10 g of each sample and then was shaken in an automatic shaker during 15 min at 150 rpm	Spectrophotometry	PCA, PLS-DM	Classification	-	[13]
Sudan I - IV	Chili powder	0.6 g of commercial chili powder was extracted with 30 mL of ethanol, and then was stirred for 10 min and sonicated for 30 min. After being precipitated at room temperature for 20 min, 2 mL of the supernatant fluid was transferred into 4 mL plastic tube and centrifugation was carried out for 6 min at 8000 rpm	Fluorescence	-	Quantification	Nanosensor based on quenching effect of hexadecyl trimethyl ammonium bromide (CTAB) stabilized upconversion nanoparticles (UCNPs) caused by the Sudan I - IV	[25]
Sudan I	Turmeric, curry, caviar, mussels and fish	2.0 mL of ethanol was added to 1.0 g of each sample and samples were incubated under ultrasonic treatment within 3 h	Lateral flow immnunoassay	-	Quantification	Use of specific monoclonal antibody conjugated with gold nanoparticle. The non-significant impact of Sudan II and IV	[45]
Sudan I	Chili powder, chili sauce and tomato sauce	6.0 g of sampled were spiked with different Sudan I concentrations, mixed with ethanol and sonicated. After that, carbon quantum dots were mix with samples	Fluorescence	-	Quantification	Emission spectra were measured after 30 min. Method based on the quenching effect caused by Sudan I in carbon quantum dots	[23]
Sudan I and IV	Paprika	Extraction with acetonitrile	Spectrophotometry	PLS-DA	Classification	Parameters were maintained for the multivariate methods throughout the 6 months of the study	[17]

Table 1. Analytical methods for the analysis of Sudan dyes in different foods.

Analyte	Matrix	Sample treatment	Analytical techniques	Chemometric algorithms	Classification/ Quantification	Other details	Ref.
Sudan I	Chili sauce, chili powder and tomato sauce	Extraction with methanol by sonication for 20 min, followed by centrifugation at 12000 rpm for 6 min	SERS	-	Quantification	ICA employing gold-silver core-shell bimetallic nanorods for immobilization of polyclonal antibody against Sudan I	[2]
Sudan I	Paprika powder	Non-destructive analysis	Raman spectroscopy	PLS, PLS-DA	Classification and quantification	Conventional Raman spectroscopy. Correction of background fluorescence signal with the polyfit routine. Detection capability (CC_β) above 0.5 % (w/w).	[3]
Sudan I and II	Paprika powder	Extraction with acetonitrile	Spectrophotometry	PLS, PLS-DA	Classification and quantification	-	[14]

RAFA: rank annihilation factor analysis ; PARAFAC: parallel factor analysis; KNN: K-Nearest Neighbor ; SIMCA: Soft Independent Modelling of Class Analogy; PLS-DA: Partial Least-Squares discriminant-analysis ; HPLC: high performance liquid chromatography; UV: ultraviolet; LOD: limit of detection; LOQ: limit of quantification ; PDS: Piecewise Direct Standardization; IAC: Immunoaffinity chromatography; MMSPD: micro-matrix solid-phase dispersion; SERS: Surface-enhance Raman Spectroscopy; ICSF: ; NIR: near-infrared; AdSV: Adsorptive stripping voltammetry; PLS-DM: partial least squares-density modeling; DPV: differential pulse voltammetry ; MCR-ALS: multivariate curve resolution - alternating Least-Squares; CV: cyclic voltammetry.

Table 2. Description of samples employed in this study.

Sample ID	ASTA value	Origin
PDO1	149	Spanish PDO "Pimentón de La Vera"
PDO2	25	
PDO3	127	
PDO4	42	
PDO5	133	
PDO6	84	
SM1	55	Spanish market
NM1	85	Norwegian market
NM2	42	
NM3	120	

397

Table 3. Composition of samples for calibration and validation sets.

Calibration set 1		
Sample ID	**ASTA value**	**Sudan I concentration (mg/g)**
PDO1	149	0
		0.4
		0.9
		2.5
		8.9
		17.8
		23
Calibration set 2		
Sample ID	**ASTA value**	**Sudan I concentration (mg/g)**
PDO2	25	0
PDO1	149	0
NM1	85	0
PDO3	127	0
NM2	42	0
PDO2	25	16.0
PDO1	149	18.0
NM1	85	0.28
NM1	85	25.2
NM2	42	3.5
PDO3	127	3.7
NM2	42	21.2
PDO3	127	21.5
NM1	85	12.6
Validation set		
Sample ID	**ASTA value**	**Sudan I concentration (mg/g)**
PDO4	42	10.8
PDO5	133	11.8
PDO6	84	2.63
PDO6	84	20.2
SM1	55	5.11
NM3	120	4.03
SM1	55	16.9
NM4	120	15.4
PDO6	84	11.1

398

Table 4. Results obtained for calibration models and test samples with the different algorithms assayed.

	1st Calibration set				Validation set		
Algorithm	**nº comp**	**R^2**	**RMSEC (mg/g)**	**REP (%)**	**R^2**	**RMSEP (mg/g)**	**REP (%)**
PLS	3	0.9838	1.1	14	0.8048	3.8	35
U-PLS	2	0.9772	1.3	17	0.7646	4.5	50
N-PLS	2	0.9778	1.3	17	0.7718	4.4	49
	2nd Calibration set				**Validation set**		
Algorithm	**nº comp**	**R^2**	**RMSEC (mg/g)**	**REP (%)**	**R^2**	**RMSEP (mg/g)**	**REP (%)**
PLS	2	0.9650	1.7	40	0.7976	5.1	45
U-PLS	5	0.9813	1.4	16	0.8850	3.0	26
N-PLS	5	0.9859	1.4	17	0.8470	2.5	25

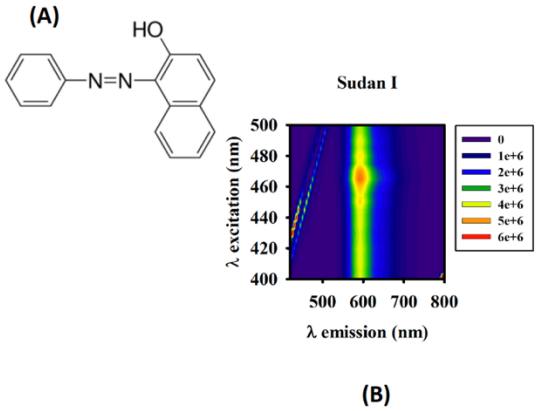
RMSEC: root mean squares error of calibration; RMSEP: root mean squares error of prediction; REP: relative error of prediction.

Figure captions:

Figure 1. (A) Structure of Sudan I compound and (B) Excitation - emission matrix for Sudan I standard obtains directly from powder standard.

Figure 2. A) Emission spectra for a paprika sample (PDO1) adulterated with different Sudan I concentrations (exc: 465 nm). B) Relationship between Sudan I concentration and fluorescence emission intensity at 588 nm.

Figure 3. Excitation - emission matrices obtained for two different paprika samples (PDO3 and PDO4) unadulterated and adulterated with different concentrations of Sudan I.



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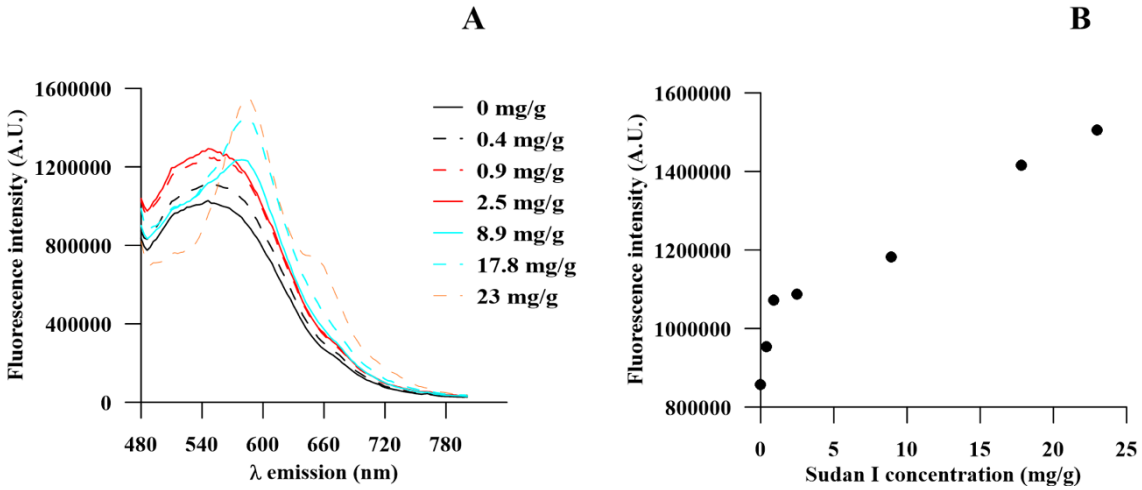
416 **Figure 1**

417



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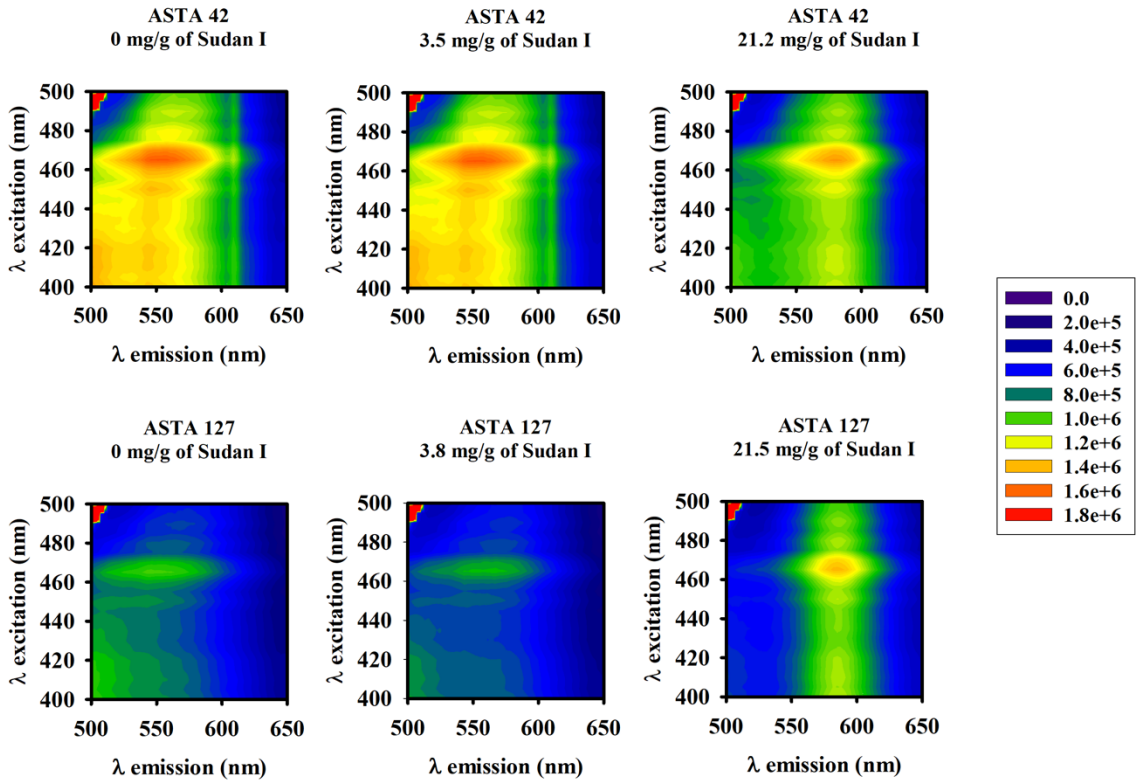
419 **Figure 2**



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421 **Figure 3**

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423