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SECOND-ORDER CALIBRATION IN COMBINATION WITH FLUORESCENCE FIBRE-OPTIC DATA MODELLING AS A NOVEL APPROACH FOR MONITORING THE MATURATION STAGE OF PLUMS

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17 **Abstract**

18 In this work, non-destructive autofluorescence of plums was employed to study the
19 chlorophylls' concentration evolution along the maturation process. For that, excitation-
20 emission matrices (EEMs), containing full fluorescence information, were collected with
21 a fibre-optic, assembled to a spectrofluorometer. Data analysis was performed with
22 several second-order multi-way algorithms, such as parallel factor analysis (PARAFAC),
23 multi-way partial least-squares (N-PLS), unfolded partial least-squares (U-PLS), and
24 multivariate curve resolution-alternating least-squares (MCR-ALS). Firstly, the EEMs of
25 each plum, collected each week along the maturation process, were processed with
26 PARAFAC. Two components were used to model the data and the excitation and
27 emission loadings were obtained. Score values for the first PARAFAC component
28 showed a clear evolution with time, increasing during the first five weeks, and decreasing
29 for the last weeks. Also, the chlorophyll concentrations obtained by HPLC analysis, in
30 the skin and the whole fruit, were compared with those obtained with different algorithms
31 mentioned before. Best results were obtained in the case of skin for all algorithms. Similar
32 correlation coefficients (r) were obtained in all cases (0.899 (PARAFAC); 0.940 (U-
33 PLS); 0.936 (N-PLS) and 0.958 (MCR-ALS)). When the elliptical joint confidence region
34 (EJCR), for the slope and intercept, were calculated, the theoretically expected values of
35 1 and 0, for the slope and intercept, respectively, were included in all ellipses. However,
36 it was observed that for the skin data and U-PLS and N-PLS algorithms, the EJCR
37 confidence region was smaller than in the other cases.

38 **Keywords:** Fibre-optic; plums; autofluorescence; second-order algorithms.

39 **1. Introduction**

40 Nowadays, the use of non-invasive approaches to characterize solid samples is being a
41 successful alternative in any environment. Among others, the autofluorescence of foods
42 obtaining fluorescence data with a fibre-optic has drawn attention, due to the fact that a
43 previous extraction process is not necessary, and short response times and minimal
44 instrumentation requirement are required.

45 The multidimensional nature of photoluminescence makes fluorescence spectroscopy an unique
46 potential for simultaneous characterization of multiple fluorescence components in complex
47 matrices. Nevertheless, to support this multidimensional information, chemometric techniques
48 are necessary to process and model the fluorescence data sets, in order to extract the highest
49 possible information content. Methods of classification, modelling, multivariate regression,
50 similarity analysis, principal components analysis, experimental design and optimization, have
51 been applied in different fields, such as environmental [1], food control [2] or medical and
52 biotech processes [3–5], among others.

53 A common non-destructive technique widely employed for quality assessment of foods and
54 agricultural products has been Near-Infrared Spectroscopy (NIRs). The benefit of this technique
55 is due to the rapid, non-destructive and low-cost analysis [6]. The first-order data obtained with
56 NIRs are mostly processed with partial least-squares regression (PLSR), being widely applied
57 in food and in agriculture analysis [7–10]. NIR absorption spectra approximately describe the
58 aggregate effect of absorption and scattering in food samples; they do not offer separate
59 information on the absorption and emission properties. Hence, NIRs, in essence, is an empirical
60 technique that relies on statistical methods to relate spectral features to the chemical or physical
61 attributes of food samples. Because of its empiricism, conventional NIR measurements are not
62 the most adequate for quantitative analysis. Other important trouble in NIRs analysis of solid

samples is that the spectral variations may be due to physical phenomena, such as dispersion, and not related with sample chemical information [11]. For last, the first-order data obtained with these techniques may not be enough to characterize complex matrices.

With the object of obtaining more selective information for the evaluation of agricultural products using non-invasive techniques, artificial noses, tongues and sensors based in fibre-optics have been developed, and the complex information provided by these instruments only can be interpreted as useful information by means of chemometric tools [12]. Hence, the combination of autofluorescence data, obtaining with a fibre optic probe, with chemometrics has been probed as a useful tool to characterize multiple fluorescent components in intact sample, allowing on-line monitoring for an appropriate quality control.

In this context, numerous studies have been performed to assess fruit harvesting time and to study the evolution of the pigments along the maturation process [13]. The most visible and frequent change in maturing fruits is the loss of green colour due to chlorophyll degradation. Plums are climacteric fruits in which the chlorophyll content decreases during ripening, and the measurement of this change is an unequivocal indication of maturation [14]. The chlorophylls determination involves tedious treatments of the sample such as several extraction steps, under dimmed light to prevent isomerization and photodegradation of pigments. These processes are time-consuming and require specialized sample preparation [15,16].

Few studies can be found in the literature where fluorescence has been employed for the estimation of maturity. For example, this technique was used to estimate the maturity of citrus using deep learning [17] or for assessment of winegrape phenolic maturity [18]. Also, chlorophyll fluorescence has been employed as non-destructive method to assess maturity of mango fruits [19] and the chlorophyll fluorescence was measured with a hand-held multi-parametric fluorescence sensor to follow the maturation of plums [20]. However, a different

87 variety was used in that study. There are no studies where the full information provided by
88 EEMs was employed for this purpose.

89 The objective of this work is to show the usefulness of chemometrics for the control of
90 maturation process of plums, without treatment of the sample, using well-established
91 chemometric tools available to any user. For that, EEMs were analysed with different purposes:
92 exploratory analysis and quantification of chlorophylls. Different algorithms were used for that:
93 parallel factor analysis (PARAFAC) [21] for exploratory analysis, and PARAFAC, multiway
94 partial least-squares (N-PLS) [22], unfolded partial least-squares (U-PLS) [23], and
95 multivariate curve resolution-alternating least-squares (MCR-ALS) [24] for quantification.

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97 **2. Materials and Methods**

98 *2.1. Reagents, solvents and standards*

99 Chlorophylls *a* and *b* (chl *a* and chl *b*) were obtained from Sigma-Aldrich Chemical Co. and
100 used as received. Stock solutions of chlorophylls a and b were prepared by dissolving the
101 ampules content (1 mg of each chlorophyll) in 25.0 mL of acetone and stored at −4 °C in
102 darkness until use. Working solutions were prepared by dilution of the appropriate aliquots with
103 acetone. Acetone was purchased from Merck (Darmstadt, Germany) and methanol (MeOH),
104 acetonitrile (ACN), both of HPLC-grade, were purchased from Panreac (Barcelona, Spain). A
105 methanolic solution of 5 mM ammonium acetate was prepared by dissolving a suitable amount
106 of ammonium acetate in methanol.

107 *2.2. Sampling*

108 The effect of maturity was studied with *Friar* Plums variety. Plums sampling was carried out
109 in a cultivar located in Badajoz, Extremadura, Southwest of Spain. Fruits were harvested each

110 week, from last week of May to August 2018. Samples were randomly collected and, for each
111 week along the maturation process, four fruits were analyzed. Firstly, the EEMs of each one
112 were recorded and, after that, the skin and the whole fruit were treated to extract the
113 chlorophylls.

114 *2.3. Excitation-emission matrices (EEMs) collection*

115 For each plum randomly collected each week, four EEMs were collected in four spots, in order
116 to have an average of the whole fruits. Hence, measurements were obtained from peduncle
117 zone, from the bottom and from other two plum faces. All measurements were obtained with
118 direct contact between the fibre and the fruits under normal laboratory illumination with a Cary
119 Eclipse spectrofluorimeter, where a fibre-optic was assembled (Agilent Technologies, Madrid,
120 Spain). The equipment was connected to a PC microcomputer via an IEEE 488 (GPIB) serial
121 interface. The Cary Eclipse 1.2 software was used for data acquisitions. EEMs were recorded
122 as a set of fluorescence emission spectra over a range of excitation wavelengths. The excitation
123 wavelengths ranged was from 360 to 500 nm in 5 nm increments. At each excitation
124 wavelength, the emission spectra were recorded from 600 to 700 nm, at 1 nm intervals. The
125 slits of excitation and emission monochromators were set at 2.5 and 5 nm, respectively. The
126 photomultiplier tube sensitivity was 700 V and the scan rate was set at 300 nm min^{-1}. The total
127 scanning time per sample was approximately 5 min.

128 *2.4. Softwares for data modelling*

129 All calculations were carried out in Matlab (Matlab R2007b, version 7.5.0.342). Routines for
130 PARAFAC were available on the internet thanks to Bro (http://www.models.kvl.dk/source/).
131 MVC2, a useful Matlab graphic interface (http://www.iquir-conicet.gov.ar/descargas/mvc2.rar)
132 was used for PARAFAC, U-PLS, N-PLS and MCR-ALS calculations [25,26].

133 *2.5. Extraction and HPLC analysis of chlorophylls*

134 For samples picked each week, the skin and whole fruit were independently analysed. The skin
135 of plums from fresh fruits was quickly removed and immediately treated. For the analysis of
136 whole plums, seeds were removed just before homogenization during 20 s with a mill. In all
137 cases, adequate weights (4 g of skin or 10 g of whole fruit) were extracted three times with 15
138 mL of THF:MeOH (1:1, v:v), in presence of BHT 0.1%, and 1 g of magnesium carbonate, with
139 continuous stirring. The supernatants were filtered under vacuum through quantitative filter
140 paper nº 1242 (Filter-Lab, Anoia, Barcelona, Spain) on a Büchner funnel. The combined
141 supernatants were re-extracted three times with 15 mL of petroleum ether and 15 mL of 10%
142 NaCl. The combined organic phases were evaporated to dryness (35 ºC in a rotatory
143 evaporator), and the residue dissolved in 5.0 mL of THF:MeOH (1:1, v:v). The measurements
144 of chlorophylls were carried out by diluting each aliquot (1:250). The extracts were filtered
145 with 0.25 μm diameter Chromafil filters (Düren, Germany), prior to the injection into the HPLC
146 system (UFLC Shimadzu Prominence LC-AD) and using a modification of the method
147 proposed by Orazem et al [27]. The analytical column was a Kinetex C18 (150 x 4.6 mm, 5
148 μm), with an analytical temperature of 30 ºC. The injection volume was 5 μL. The mobile phase
149 consisted on acetonitrile:methanolic solution of ammonium acetate (5 mM), 95:5 (v/v), with a
150 flow-rate of 1.0 mL min^{-1}. A fluorimetric detector was used, and 660 and 420 nm were set for
151 emission and excitation wavelength, respectively. External standard calibration, based on peak
152 areas, was used for quantification, and the concentrations of chlorophyll pigments were
153 determined in whole fruit and in the skin.

154 **3. Results and discussion**

155 *3.1. Excitation-emission matrices description of intact plums*

156 In order to obtain the fluorescence fingerprints from intact plums, EEMs were collected with a
157 fibre-optic along nine weeks from May to August 2018. The advantage of using a fibre-optic is
158 that it allows obtaining fluorescence information of fruits directly on the tree or in the packaging
159 conveyor belt. The conditions employed to collect the EEMs were as described in section 2.3.

160 In Figure 1, the EEMs of samples harvested the first and the last sampling weeks and some
161 pictures of samples from these weeks are illustrated. As can be observed, during the first weeks,
162 samples exhibited high fluorescence signals at emission wavelengths between 670 and 700 nm,
163 with high excitation signals between 400 and 500 nm. This fluorescent signal decreases as
164 plums maturation takes place, and it is not appreciated practically after the week number eight.
165 Visually, the change of colour is also appreciated in the pictures.

166 The advantage of using fluorescence signals as fingerprint of samples is related with its high
167 selectivity and sensitivity. Three-dimensional map of samples offers a huge information of
168 complex samples as foods are. Furthermore, the obtention of the EEMs with a fibre-optic avoids
169 the sample treatment and allows on-line monitorization of samples for an appropriate quality
170 control.

171 *3.2. Qualitative study about the maturation process of plums*

172 Firstly, a previous qualitative study about the maturation process was performed. For that,
173 EEMs of the plums, recollected each week, during a period of nine weeks, were separately
174 examined. A different 3D data set for each week was built, resulting 12 EEMs, corresponding
175 to three plums samples and, for each plum, four EEMs obtained in different faces of the fruit.

176 PARAFAC was applied independently in the 12 EEMs of each week. The data were arranged
177 in 3D arrays with dimensions 12 x 101 x 29 (samples (3 plums x 4 faces of each plum) x number
178 of emission wavelengths x number of excitation wavelengths). These arrays were decomposed

179 by PARAFAC [21], applying the core consistency diagnostic (CORCONDIA) [28], analysing
180 the residuals [29], and evaluating the shape profile of the loadings, for optimization of the
181 number of components [26]. The core consistency analysis consists on studying the structural
182 model based on the data and the estimated parameters of gradually augmented models. A model
183 is considered to be appropriate if adding other combinations of components does not improve
184 the fit considerably [28]. On the other hand, the analysis of residuals considers the residual fit
185 of the PARAFAC model as a function of increasing number of factors. The appropriate model
186 is the one which is not statistically different from the model leading to the minimum residual
187 fit [26,29]. For the 3D array of each week, different numbers of components were assayed (from
188 1 to 5). In all cases, non-negative constraints, for the resolved profiles in all modes, were
189 applied, with the purpose of obtaining a realistic solution, because concentrations and spectral
190 values cannot be negative.

191 In Table 1, core consistency values, explained variance and the standard deviation of residuals
192 with the number of components are shown. The optimum number of components for each week
193 are bold remarked. As appreciated, during the first six weeks, and using the core consistency
194 criterion, the optimal number of components was two. This optimization was performed
195 considering that the optimal number of components is selected as the largest tested value for
196 which the core consistency is larger than ≈50%. For the first six weeks, when the number of
197 components goes from two to three, the core consistency percentage falls from 100% to about
198 40%. The SD residual values decrease from 3 to 1, approximately, when the number of
199 components goes from one to two and then, the value was stable from two to five. Hence, with
200 this criterion, the optimal number of components was also two. For the explained variance, it is
201 appreciated that in all cases is higher than 99 % when two components are used as optimal.

202 In the Figures 2A, 2B and 2C it can be seen the excitation-emission profiles retrieved from
203 PARAFAC for different weeks (week 2, week 4 and week 7). The excitation and emission
204 loadings of the first components show very similar profiles along the weeks sampling. The first
205 component shows a very clear and defined shape with an emission maximum at 685 nm, and
206 two excitation maxima at 440 and 485 nm. Regarding to the second component, its emission
207 profile does not show relevant information, however, the excitation profile is well-defined with
208 a maximum at 395 nm and it slightly increases when the maturation process does it.

209 From seventh week, when PARAFAC was applied to the recorded EEMs, the number of
210 components increased as indicated by the core value, which falls from 60% to 20%
211 (approximately) when the number of components goes from three to four (Table 1). Also,
212 increasing the number of components the value for the explained variance is similar to previous
213 weeks. The excitation and emission profiles of the three components are represented in Figure
214 2C. The shape of the two first components are similar to those obtained along the first six weeks,
215 and the third component shows a well-defined excitation maximum at 400 nm, and two
216 emission maxima at 600 nm and 650 nm. However, it was difficult the identification of this
217 component.

218 After this, taking into account the variability of different EEMs along the maturation process,
219 different 3D arrays were obtained with the objective of reducing that variability. The first 3D
220 array was from plums (average of four measurements in different faces) of each week, resulting
221 a structure with dimensions 27 x 101 x 29 (samples (3 plums x 9 weeks) x number of emission
222 wavelengths x number of excitation wavelengths). As in previous case, different criteria were
223 used to select the optimal number of components and two were enough to explain 99.4 % of
224 the variance. Figure 3A shows the score values for the first and second components with the
225 time. Score values for the first component increase during the first weeks and then decrease

226 from the sixth week. Scores values for the second component appear quite constant along the
227 time, a clear trend is not observed in this case.

228 Secondly, another data set was obtained from the average of all EEMs from each week. In this
229 case, the number of samples was reduced to 9, corresponding with the number of weeks that
230 samples were recollected. As appreciated in Figure 3B, the evolution of score values for the
231 first component shows that it increases along the three first weeks, after that, it is constant for
232 two weeks and, then, it starts to decrease until it is almost zero.

233 According to the excitation and emission wavelengths of the loading profiles for the first
234 component, this component could be related with the chlorophyll compounds present in plums.
235 In the literature, the excitation/emission maxima for the chlorophyll are 458/653 nm when the
236 spectra were register in acetone/water medium [30]. The position of the maximum can shift to
237 different wavelengths when spectra are obtained from intact solid samples rather from a
238 solution due to the variation that molecules could suffer, as we proved in another study with
239 other compounds [31].

240 For this reason, a quantification of these compounds was performed by HPLC-FLD, as detailed
241 in the next section. Taking into account that the best results were obtained when using the
242 average of samples from each week, these data were used in the following section.

243 *3.3. Quantification of chlorophylls with second-order algorithms*

244 Once a previous identification of PARAFAC components on the basis of the fluorescence
245 spectrum was done, it was interesting to confirm this assumption evaluating the relationship
246 between score values obtained by PARAFAC for the first component and the concentration of
247 chlorophyll. The chlorophyll concentrations were obtained by HPLC-FLD in extracts of plums
248 recollected along the nine weeks, in both, skin and the whole fruit. Firstly, in the case of skin

249 chlorophyll content, when the score values of the first PARAFAC component were related with
250 chlorophyll concentration values, a correlation coefficient of 0.8998 was found. In the case of
251 whole fruit, this coefficient was lower (r = 0.8626).

252 In order to compare these results with those obtained with other algorithms, MCR-ALS, U-PLS
253 and N-PLS were applied. In all cases, the data were arranged in a 3D array with dimensions 9
254 x 101 x 29 (samples (average values in each of the 9 weeks) x number of emission wavelengths
255 x number of excitation wavelengths). The first step when using MCR-ALS was to obtain the
256 augmented matrices in the excitation wavelength direction. The number of components in each
257 augmented matrix was estimated by principal component analysis (PCA), and justified taking
258 into account the presence of the corresponding analytes, possible interferences, and background
259 signals. Non-negativity restriction was applied in both modes, emission and excitation
260 spectroscopic spectral data, and unimodality restriction was applied only to the signals
261 corresponding to the analytes, and not to the background signals. After ALS optimization for
262 each sample, and with the aid of the corresponding pseudounivariate calibration curves, the
263 constituents were identified and quantified. The optimal number of components was 3, which
264 explained the 99.7 % of variance. In Figure 4, excitation profiles retrieved by MCR-ALS and
265 the emission spectra for each component are shown. A comparison of the profiles of the first
266 component with those obtained by PARAFAC, indicates that the excitation and emission
267 maxima are very similar (Pearson regression coefficients of 0.9890 and 0.9637, for emission
268 and excitation, respectively, and with 95 % of level confidence were calculated). The
269 correlation coefficient between the score values of the first component and the HPLC data was
270 0.9581, when the score values of the first component *versus* the measured HPLC content of
271 chlorophyll in the skin, were plotted, and 0.8851 for the whole fruit content.

272 Furthermore, U-PLS and N-PLS were assayed. In these cases, the Haaland and Thomas
273 criterion [32,33] was employed to select the number of optimal components, which are those
274 given a PRESS value statistically no different to the minimum PRESS value (F-ratio probability
275 falling below 0.75). The optimum number of latent variables was three. When concentrations
276 of chlorophyll were predicted by both algorithms, the correlation coefficients between predicted
277 and true concentration were 0.9400 and 0.9360 for U-PLS and N-PLS, respectively, for the
278 concentration in the plums' skin, and 0.8770 and 0.9091, respectively for concentration in
279 whole fruits. Figures 5A and 5C show the plots obtained for all algorithms between true and
280 predicted concentrations.

281 In Table 2, all figures of merit are included. It can be appreciated that the best results were
282 obtained in the skin and for the U-PLS and N-PLS algorithms because the root mean square
283 error of predictions (RMSEPs) and the relative error of predictions (REPs) are lower in these
284 cases.

285 Finally, the elliptical joint confidence region EJCR test (at 95% confidence level) [34] was
286 applied for the different algorithms to evaluate the slope and intercept, corresponding to the
287 linear regression of predicted concentrations for the algorithms vs the HPLC concentrations.
288 The corresponding ellipses are shown in Figures 5B and 5D, for the skin and whole fruit results,
289 respectively. Note that in all cases the critical point (1,0) is included, which proved the accuracy
290 between true and predicted concentrations for the different algorithms. However, in the case of
291 the skin analysis, the ellipses for U-PLS and N-PLS are smaller in size, suggesting higher
292 precision for these algorithms.

293 As conclusion, it can be said that the results were quite similar for all algorithms, and better in
294 the case of the skin results. From the results obtained in this study, it can be highlighted that
295 second-order algorithms are a powerful tool for the characterization of agronomic processes.

Furthermore, the combination with a non-destructive technique, such as a fibre-optic, and using a selective signal, as autofluorescence of the sample, is another step in the possible automatization of the maturation process.

4. Conclusions

Non-destructive characterization of fresh plums was performed for the first time, employing fibre-optic data with second-order calibration. Results showed that chlorophyll content could be a good indicator of maturation process. Taking into account that timing of fruit picking (harvest maturity) significantly impacts in the postharvest handling systems, especially when international deliveries are performed, it is very important to dispose non-destructive techniques that allow deciding the harvest date. Moreover, fluorescence fingerprints in combination with second-order calibrations can be a powerful tool for determination of chlorophyll content in plums.

Although the best results are obtained with U-PLS and N-PLS in the skin of plums, as indicate the smaller size of EJCR regions, it is interesting the possibility that PARAFAC and MCR-ALS offer to be able to obtain spectral information about the fluorescence components.

The obtained results seem very promising and which could be used as references of maturation in situ, employing a fibre-optic probe with a portable system. However, more samples will be necessary to expand the calibration data set to develop a robust prediction model that can be used in practice.

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References

[1] L.L. Hohrenk, M. Vosough, T.C. Schmidt, Implementation of Chemometric Tools To Improve Data Mining and Prioritization in LC-HRMS for Nontarget Screening of Organic Micropollutants in Complex Water Matrixes, Anal. Chem. 91 (2019) 9213–9220.

[2] F. Marini, Chemometrics in Food Chemistry, in: F. Marini (Ed.), Elsevier, 2013.

[3] A.S. Rathore, N. Bhushan, S. Hadpe, Chemometrics applications in biotech processes: A review, Biotechnol. Prog. 27 (2011) 307–315.

[4] J. Mocak, Chemometrics in Medicine and Pharmacy, Nov. Biotechnol. Chim. 11 (2012) 11–26.

[5] S.M. Faassen, B. Hitzmann, Fluorescence spectroscopy and chemometric modeling for bioprocess monitoring, Sensors (Switzerland). 15 (2015) 10271–10291.

[6] F. Zhu, H. Yao, Z. Hruska, R. Kincaid, R. Brown, D. Bhatnagar, T. Cleveland, Integration of fluorescence and reflectance visible near-infrared (vnir) hyperspectral images for detection of aflatoxins in corn kernels, Trans. ASABE. 59 (2016) 785–794.

[7] J.U. Porep, D.R. Kammerer, R. Carle, On-line application of near infrared (NIR) spectroscopy in food production, Trends Food Sci. Technol. 46 (2015) 211–230.

[8] V. Cortés, J. Blasco, N. Aleixos, S. Cubero, P. Talens, Monitoring strategies for quality control of agricultural products using visible and near-infrared spectroscopy: A review, Trends Food Sci. Technol. 85 (2019) 138–148.

[9] Y. Uwadaira, Y. Sekiyama, A. Ikehata, An examination of the principle of non-

destructive flesh firmness measurement of peach fruit by using VIS-NIR spectroscopy, Heliyon. 4 (2018).

[10] J.G. Kim, Y. Park, M.H. Shin, S. Muneer, R. Lerud, C. Michelson, D. Il Kang, J.H. Min, H.M.P.C. Kumarihami, Application of NIR-Spectroscopy to predict the harvesting maturity , fruit ripening and storage ability of Ca-chitosan treated baby kiwifruit, J. Stored Prod. Postharvest Res. 9 (2018) 44–53.

[11] D. Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., J. Jong, S., Lewi, P.J., Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Elsevier, Amsterdam, 1997.

[12] R. Guidetti, R. Beghi, V. Giovenzana, Chemometrics in Food Technology, in: K. Varmuza (Ed.), Chemom. Pract. Appl., IntechOpen, 2012.

[13] M. Bajcar, B. Saletnik, M. Zardzewiały, B. Barbara Drygaś, M. CzernMiłoszicka, C. Puchalski, Z. Grzegorz, Method for Determining Fruit Harvesting Maturity, J. Microbiol. Biotechnol. Food Sci. 6 (2016) 773–776.

[14] C.H. Crisosto, Stone fruit maturity indices: a descriptive review, Postharvest News Inf. 5 (1994) 65N-68N.

[15] B. Schoefs, Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis, Trends Food Sci. Technol. 13 (2002) 361–371.

[16] R. Delgado-Pelayo, L. Gallardo-Guerrero, D. Hornero-Méndez, Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties, Food Res. Int. 65 (2014) 272–281.

[17] K. Itakura, Y. Saito, T. Suzuki, N. Kondo, F. Hosoi, Estimation of citrus maturity with fluorescence spectroscopy using deep learning, Horticulturae. 5 (2019) 1–9.

[18] Z.G. Cerovic, N. Moise, G. Agati, G. Latouche, N. Ben Ghozlen, S. Meyer, New

portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence, J. Food Compos. Anal. 21 (2008) 650–654.

[19] M. Lechaudel, L. Urban, J. Joas, Chlorophyll fluorescence, a nondestructive method to assess maturity of mango fruits (Cv. 'cogshall') without growth conditions bias, J. Agric. Food Chem. 58 (2010) 7532–7538.

[20] A. Salama, M. Neumüller, D. Treutter, Preliminary study on non-destructive assessment of european plum (prunus domestica L.) maturity, Acta Hortic. 1099 (2015) 257–262.

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

[22] W. Rose, Principal component analysis: Principal component analysis, Wiley Interdiscip. Rev. Comput. Stat. 2 (2010) 433–459.

[23] R. Bro, Multiway calibration. Multilinear PLS, J. Chemom. 10 (1996) 47–61.

[24] R. Tauler, Multivariate curve resolution applied to second order data, Chemom. Intell. Lab. Syst. 30 (1995) 133–146.

[25] A.C. Olivieri, H.-L. Wu, R.-Q. Yu, MVC2: A MATLAB graphical interface toolbox for second-order multivariate calibration, Chemom. Intell. Lab. Syst. 96 (2009) 246–251.

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

[27] P. Orazem, M. Mikulic-Petkovsek, F. Stampar, M. Hudina, Changes during the last ripening stage in pomological and biochemical parameters of the "Redhaven" peach cultivar grafted on different rootstocks, Sci. Hortic. (Amsterdam). 160 (2013) 326–334.

[28] R. Bro, H.A.L. Kiers, A new efficient method for determining the number of components in PARAFAC models, J. Chemom. 17 (2003) 274–286.

[29] 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.

[30] T. Galeano Díaz, I. Durán Merás, C.A. Correa, B. Roldán, M.I. Rodríguez Cáceres, Simultaneous Fluorometric Determination of Chlorophylls a and b and Pheophytins a and b in Olive Oil by Partial Least-Squares Calibration, J. Agric. Food Chem. 51 (2003) 6934–6940.

[31] O. Monago-Maraña, C.E. Eskildsen, A. Muñoz de la Peña, T. Galeano-Díaz, J.P. Wold, 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, Microchem. J. 154 (2020) 104539.

[32] 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.

[33] D.M. Haaland, E. V. Thomas, Partial Least-Squares Methods for Spectral Analyses. Application to Simulated and Glass Spectral Data, Anal. Chem. 60 (1988) 1202–1208.

[34] A.G. González, M.A. Herrador, A.G. Asuero, Intra-laboratory testing of method accuracy from recovery assays, Talanta. 48 (1999) 729–736.

Figure captions

Figure 1. Fluorescence contour plots of the EEMs obtained with a fibre-optic, and pictures from samples corresponding to the first week of sampling (May 2018) and last week of sampling (August 2018).

Figure 2. Excitation and emission PARAFAC profiles of samples belonging to different maturation weeks: A) week 2; B) week 4; C) week 7.

Figure 3. Evolution of the score values for the first and second components retrieved by PARAFAC, considering the average of each plum measurement (A), and considering the average of each week (B), along the time (in weeks).

Figure 4. (A) Excitation profiles retrieved by MCR-ALS analysis for different weeks of recollection. (B) Emission spectra retrieved by MCR-ALS. Dashed lines correspond to excitation profiles and emission spectra retrieved by MCR-ALS for unknown compounds and background signals. Continuous lines correspond to excitation and emission spectra, retrieved by MCR-ALS, for chlorophyll.

Figure 5. Plots of chlorophyll predicted concentrations, for the different algorithms, as a function of the true concentrations obtained by HPLC (A and C). Corresponding elliptical joint regions (at 95 % confidence level), for the slopes and intercepts of the regressions. The theoretical point (intercept = 0; slope = 1) is marked in the figure by the black cross (B and D).

Table 1. Core, SD values and explained variance obtained along the nine weeks studied.

	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9	
Components	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**	**Core**	**SD**
1	100	2.3	100	2.3	100	2.7	100	3.1	100	3.3	100	3.1	100	2.4	100	3.1	100	2.0
2	**100**	**0.97**	**100**	**0.94**	**99**	**1.1**	**99**	**1.3**	**100**	**1.4**	**99**	**1.3**	100	1.1	99	1.4	97	1.2
3	48	0.86	43	0.80	38	0.90	33	1.1	38	1.1	33	1.1	**58**	**0.89**	**57**	**1.0**	**57**	**0.64**
4	24	0.75	9	0.71	7.7	0.74	15	1.0	23	1.0	15	1.0	18	0.81	26	0.87	4.9	0.58
5	6	0.86	3	0.64	-0.94	0.66	0.46	0.87	0.74	0.87	0.5	0.87	1.0	0.72	2.3	0.79	3.0	0.55
Explained variance (%)*	99.5		99.4		99.3		99.4		99.3		98.9		99.3		99.1		98.9	

*for optimal number of components

Table 2. Figures of merit for different algorithms assayed.

		Skin							Whole fruit						
Algorithm	**Components**	**Slope**	**Intercept**	**R^2**	**Sr**	**Test of significance**	**RMSEP (mg/g)**	**REP (%)**	**Slope**	**Intercept**	**R^2**	**Sr**	**Test of significance**	**RMSEP (mg/g)**	**REP (%)**
PARAFAC	3	1.0	-0.001	0.8097	0.2	s	0.2	26	1.0	-1e-7	0.7440	0.1	s	0.1	37
U-PLS	3	0.93	0.03	0.8842	0.1	s	0.1	20	0.67	0.09	0.7691	0.2	s	0.1	24
N-PLS	3	0.94	0.03	0.8767	0.1	s	0.1	21	1.0	-0.09	0.8256	0.2	s	0.1	38
MCR-ALS	3	1.3	-0.31	0.9180	0.8	s	0.2	44	1.1	-0.15	0.7835	0.4	s	0.1	74

s: Pearson correlation significative test (p-value < 0.05).