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Additional Information

1 **Fast detection of cocoa shell in cocoa powders by Near Infrared Spectroscopy and**  
2 **multivariate analysis**

3

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16

17 **ABSTRACT**

18 Cocoa shell must be removed from the cocoa bean before or after the roasting process. In  
19 the case of a low efficient peeling process or the intentional addition of cocoa shell to  
20 cocoa products (i.e. cocoa powders) to increase the economic benefit, quality of the final  
21 product could be unpleasantly affected. In this scenario, the Codex Alimentarius on cocoa  
22 and chocolate has established that cocoa cake must not contain more than 5% of cocoa  
23 shell and germ (based on fat-free dry matter). Traditional analysis of cocoa shell is very  
24 laborious. Thus, the aim of this work is to develop a methodology based on near infrared  
25 (NIR) spectroscopy and multivariate analysis for the fast detection of cocoa shell in cocoa  
26 powders. For this aim, binary mixtures of cocoa powder and cocoa shell containing  
27 increasing proportions of cocoa shell (up to ca. 40% w/w based on fat-free dried matter)  
28 have been prepared. After acquiring NIR spectra (1100-2500 nm) of pure samples (cocoa  
29 powder and cocoa shell) and mixtures, qualitative and quantitative analysis were done.  
30 The qualitative analysis was performed by using principal component analysis (PCA) and  
31 partial least squares discriminant analysis (PLS-DA), finding that the model was able to  
32 correctly classify all samples containing less than 5% of cocoa shell. The quantitative  
33 analysis was performed by using a partial least squares (PLS) regression. The best PLS  
34 model was the one constructed using extended multiple signal correction plus orthogonal  
35 signal correction pre-treatment using the 6 main wavelengths selected according to the  
36 Variable Importance in Projection (VIP) scores. Determination coefficient of prediction  
37 and root mean square error of prediction values of 0.967 and 2.43, respectively, confirmed  
38 the goodness of the model. According to these results it is possible to conclude that NIR  
39 technology in combination with multivariate analysis is a good and fast tool to determine  
40 if a cocoa powder contains a cocoa shell content out of Codex Alimentarius  
41 specifications.

42

43 *Keywords:*

44 Cocoa powder

45 Cocoa shell

46 NIR

47 PLS

48 PLS-DA

49 **1. Introduction**

50

51 Cocoa powder is a cocoa bean (*Theobroma cacao*) derivative largely consumed around  
52 the world due to its capacity to give color, flavor and eating pleasure to a myriad of food  
53 preparations (Dico et al., 2018).

54 The obtaining of cocoa powder from cocoa beans follows different steps. First of all,  
55 beans must be peeled, starting with the peeling of the bean before or after a roasting  
56 process. During the same peeling, cocoa cotyledon must be separated from cocoa shell  
57 (12-20% of the cocoa seed), yielding fragments of cotyledon, called nibs (Okiyama,  
58 Navarro, & Rodrigues, 2017). During the shelling step, shell should be perfectly  
59 separated, removing large parts of shells and leaving nib particles practically unbroken  
60 (Beckett, 2009). The performance of this procedure is very relevant since the presence of  
61 cocoa shell in cocoa beans derivatives (cocoa liquor, cocoa powder or chocolate)  
62 adversely affects the final product quality (Mendes & Lima, 2007). Concretely, it can  
63 have an influence in some characteristics of the final product such as the flavor or taste;  
64 it can also be responsible of off-flavors. Additionally, fiber content in cocoa shell is really  
65 high. Thus, it can be a problem for the grinding process, causing equipment abrasion in  
66 some cases. Bearing this in mind it is not surprising that shell content in cocoa powders  
67 is a quality parameter to be controlled. Concretely, the Codex Alimentarius establishes a  
68 maximum amount of 5% of cocoa shells in cocoa cake (based on fat-free dry matter)  
69 (Codex Alimentarius, 2016).

70 Analysis of cocoa shell in cocoa products might be done following the AOAC 968.10  
71 or the 970.23 methods (Codex Alimentarius, 2016). The first method, called spiral vessel  
72 count consists of counting the spiral vessels in a defatted, grinded and digested sample  
73 with the help of a microscope adjusted to mold counting (field of view 1.382 mm at 100x)

74 (AOAC, 1984). The second method, called stone cell count, consists of microscope  
75 assisted counting the stone cells present in the samples after a really laborious preparation  
76 (AOAC, 1984).

77 Since those methods are really arduous, recent attempts to develop alternative methods  
78 have been done. Researchers from the Nestlé Research Center proposed a gas-liquid  
79 chromatography procedure based on the detection of fatty acid tryptamides (FATs) in the  
80 sample, since FATs are compounds more abundant in cocoa shells than in other parts of  
81 cocoa seed. This work, carried out with only cocoa originating from the Ivory Coast,  
82 demonstrated that it might be an appropriate tool for the determination and prediction of  
83 the shell content in cocoa liquor (Hug, Golay, Giuffrida, Dionisi, & Destailats, 2006). In  
84 another work, Yang et al. (2015) proposed the employment of polysaccharide fingerprint  
85 established by high performance liquid chromatography followed by principal component  
86 analysis to identified cocoa powders adulterated with cocoa or other plant shells such as  
87 chestnut, longan, peanut, etc. However, only cocoa powders containing cocoa or other  
88 plant shell percentages higher than 15 and 10%, respectively, were detected using this  
89 methodology. Therefore, even when these methodologies (determination of FATs, HPLC  
90 polysaccharide fingerprint, etc.) are more sensible, accurate and faster than the methods  
91 proposed by the Codex Alimentarius, their use as routine techniques for shell content  
92 determination still have certain limitations such as the limit of detection or the fact that  
93 they need sample preparation, require specialized personnel and they are destructive. To  
94 avoid these drawbacks common in traditional chemical analysis techniques, recent  
95 attempts on developing accurate and sensitive analytic techniques based on near infrared  
96 (NIR) spectroscopy have been done. Due to the ability of NIR spectroscopy to provide a  
97 spectrum that acts as a ‘fingerprint’ distinctive of a particular sample, this technology is  
98 now widely used as a successful quality control tool (Lerma-García, Cortés, Talens &

99 Barat, 2018). Concretely, in the cocoa sector NIR spectroscopy has been employed for  
100 the prediction of majority (moisture, carbohydrate, fat, protein) or minority functional  
101 compounds (theobromine, catechin, organic acids, etc.) (Veselá, Barros, Synytsya,  
102 Delgadillo, Čopíková, & Coimbra, 2007; Álvarez et al., 2012; Krämer et al., 2015) as  
103 well as for quality control (discrimination of cocoa beans according to geographical  
104 origin, prediction of cocoa powder adulterations, etc) (Teye, Huang, Dai & Chen, 2013;  
105 Quelal et al., 2018).

106 In this scenario, the goal of this work is the fast determination of cocoa shell content  
107 in cocoa powders in concentrations higher than the limit established by the Codex  
108 Alimentarius (5%) by means of NIR spectroscopy and a multivariate analysis.

109

## 110 **2. Materials and methods**

111

### 112 *2.1. Cocoa powder and shell Samples*

113

114 A total of 20 natural cocoa powders and 2 cocoa shells, gently provided by Olam Food  
115 Ingredients (Cheste, Spain) or purchased in the market from different origins (Ghana,  
116 Ivory Coast, Cameroon, Peru and Indonesia) were employed in this study. In order to  
117 predict the presence of cocoa shell in cocoa powders using partial least squares (PLS),  
118 binary mixtures containing cocoa powder and cocoa shell were prepared. The mixtures  
119 contained percentages of cocoa shells in cocoa powder (based on fat-free dry matter) from  
120 ca. 2.5 to 40%. Percentages higher than 40% were not considered since over this  
121 percentage the presence of cocoa shell is sensory evident. To improve the robustness of  
122 the PLS model, all 20 cocoa powder samples (coming from different origins and obtained  
123 after different processings) were randomly selected to perform a total of 12 binary

124 mixtures for each percentage (2.5, 5, 7.5, 10, 20 and 40%), in which both cocoa shell  
125 samples were also considered. Thus, a total of 72 mixtures were obtained. Once all  
126 mixtures were prepared, they were poured in hermetic plastic containers and stored at  
127  $20\pm 2$  °C under dark conditions until use.

128

## 129 *2.2. NIR spectra acquisition*

130 The 94 samples (20 cocoa powders, 2 cocoa shells and 72 binary mixtures) were  
131 measured with a spectrophotometer FOSS NIR 5000 (Silver Spring, MD, USA). A  
132 uniform thickness and surface were secured during spectra scanning using a device with  
133 380 mm of diameter and 1cm of thick with a quartz windows which was filled with 5 g  
134 of sample. The spectrophotometer gives the measurements in relative absorbance units  
135 ( $\log 1/R$ ), which could be correlated with chemical constituents (Liu, Sun, & Ouyang,  
136 2010; Martens, Nielsen, & Engelsen, 2003). Each sample was scanned 32 times in a range  
137 comprised between 1100 and 2500 nm at 2 nm intervals (700 points). The samples were  
138 measured twice and no differences between them were found.

139

## 140 *2.3. Statistical analysis*

141

142 Spectral data were pre-treated and analysed using qualitative and quantitative models  
143 by means of the chemometric software Unscrambler v10.5 (CAMO Software AS, Oslo,  
144 Norway).

145 The PCA model was performed using raw data to identify different sample groups and  
146 to find and remove defective outliers (Adnan, Hörsten, Pawelzik, & Mörlein, 2017; Bro  
147 & Smilde, 2014).

148 The PLS was performed in order to predict the presence of cocoa shell in the cocoa



149 powders and the PLS-DA (Berrueta, Alonso, & Héberger, 2007; Prats-Montalbán, Jerez-  
150 Rozo, Romañach, & Ferrer, 2012), was constructed to evaluate its capability in  
151 classifying samples according to the following categories: cocoa powders containing less  
152 than 5% cocoa shell (w/w), and cocoa powders containing from 5 to 40% cocoa shell  
153 (w/w).

154 Both analyses were performed using the pre-treated spectra. The spectral pre-  
155 treatments tried included extended multiple signal correction (EMSC) (Martens et al.,  
156 2003), standard normal variation (SNV), 2<sup>nd</sup> derivative with the Savitzky-Golay (S-G),  
157 orthogonal signal correction (OSC) and combinations of all of them with OSC.

158 To construct both PLS and PLS-DA models, two data matrices were used. The first  
159 one employed for the PCA and PLS model construction, contained the spectra of all  
160 samples (N = 94) and the same 700 X-variables. In this case, all individual cocoa shell  
161 percentages were considered as Y-variable. The second matrix, employed for the PLS-  
162 DA model construction, included the spectra of 92 samples (in which the spectra of cocoa  
163 shells were not considered since the considered categories were cocoa shell contents  
164 below 5% and between 5-40%) and 700 predictors or X-variables (wavelengths), and also  
165 a dependent Y-variable containing the 2 categories previously described (<5% and 5-40%  
166 cocoa shell based on fat-free dry matter, w/w).

167 For both, PLS-DA and PLS models construction, the use of all spectra wavelengths  
168 was considered, jointly with the use of the most important wavelengths. The PLS and the  
169 score of Variable Importance in Projection (VIP) were combined together for these  
170 selection (Botelho, Reis, Oliveira, & Sena, 2015).

171 To select the optimal factor number and to avoid the over-fitting of both PLS and PLS-  
172 DA models, leave-one-out cross-validation was used using 70% of the data, which were  
173 randomly selected. The remaining 30% of the data were used as an external validation

174 set.

175 PLS models accuracy was evaluated by the required number of latent variables (LVs),  
176 the coefficient of determination of calibration ( $R^2_C$ ), RMSEC, the coefficient of  
177 determination of cross-validation ( $R^2_{CV}$ ), RMSECV, the coefficient of determination for  
178 prediction ( $R^2_P$ ), the root mean square error of prediction (RMSEP), the ratio of prediction  
179 deviation (RPD, which is calculated as ratio between the standard deviation of reference  
180 values in training set and RMSEP) and the bias value (which establishes the difference  
181 between experimental values and NIR predictions). Bias value can be positive  
182 (overestimating) or negative (underestimating), indicating values near to zero a minimum  
183 deviation from experimental and predicted values (Cantor, Hoag, Ellison, Khan, & Lyon,  
184 2011).

185 On the other hand, the number of latent variables (LVs) for the PLS-DA model was  
186 determined by the low value of the root mean square error of calibration (RMSEC), and  
187 the root mean square error of leave-one-out cross validation (RMSECV) (Botelho et al.,  
188 2015). The PLS-DA classification performance was evaluated by sensitivity, specificity  
189 and by the non-error rate (NER). Sensitivity is the model ability related to a correct  
190 classification of the samples with different levels of cocoa shell content. The model  
191 capacity to correctly determine the samples which not correspond to the class and  
192 correctly refuse them is the specificity (Almeida, Fidelis, Barata, & Poppi, 2013). The  
193 non-error rate (NER) is the average of the sensitivities of the different categories  
194 (Manfredi, Robotti, Quasso, Mazzucco, Calabrese, & Marengo, 2018).

195

196

197

198

199 **3. Results and discussion**

200

201 *3.1. Cocoa powder and shell spectra, pre-treated spectra and PCA analysis*

202

203 The mean raw spectra of cocoa powders, cocoa shells and binary mixtures of them at  
204 different percentages are shown in Fig. 1a. As shown in this figure, the main bands  
205 observed appeared at 1470, 1930 and 2130 nm, although other bands at 1730, 2310 and  
206 2350 nm were also evidenced. Although all spectra have a similar pattern of absorbance,  
207 the relative absorbance of these bands is different for the different types of samples: cocoa  
208 shell is characterized by the highest relative absorbance, which decreased when the  
209 content of cocoa shell in the samples decreased. The signal at 1470 nm correspond to the  
210 first overtone of O-H and N-H stretching which is associated with a CONH<sub>2</sub> structure  
211 (peptide) and related to a protein (Osborne, Fearn, & Hindle, 1993). The signal at 1930  
212 nm is related with asymmetric stretching and rocking of water, weakly bounded water,  
213 proteins, and aromatics (Veselá et al., 2007), while the wavelength at 2130 nm can be  
214 assigned to N-H combination bands (CONH<sub>2</sub>) (Ribeiro, Ferreira, & Salva, 2011). On the  
215 other hand, the band at 1730 nm could be assigned to the first overtone of C-H (Ribeiro  
216 et al., 2011), while 2310 and 2350 nm are mostly related to stretching and rocking  
217 vibrations of CH<sub>2</sub> of polysaccharides (Veselá et al., 2007).

218

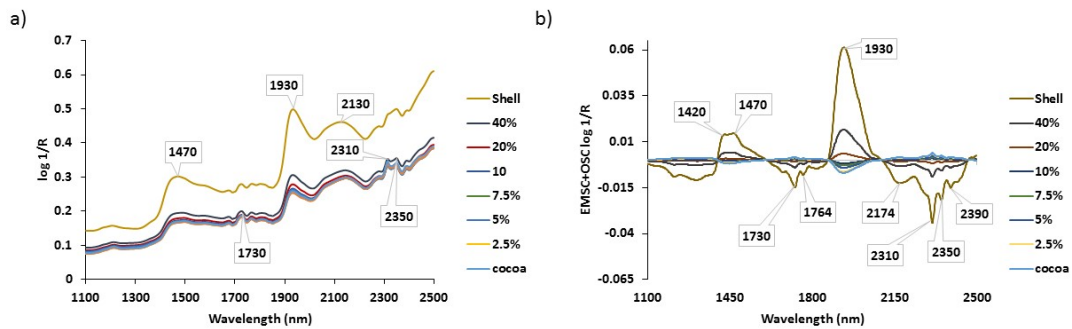


Fig 1. M.A. Quelal-Vásquez et al.

219

220 **Fig 1.** Mean spectra of cocoa powders and shells and mixtures of them at different  
 221 percentages from (a) raw and (b) pre-treated with EMSC-OSC spectra.

222

223

224 The mean spectra obtained after the application of the EMSC-OSC pre-treatment is  
 225 shown in Fig. 1b. In this case, the principal wavelengths were 1420, 1470, 1730, 1764,  
 226 1930, 2174, 2310, 2350 and 2390 nm. Most of the bands have been previously described,  
 227 while the other ones could be attributed to the first overtones of symmetric and anti-  
 228 symmetric C-H stretch vibration (CH<sub>2</sub>-groups) (1764 nm) (Krähmer et al., 2015), to a  
 229 combination of C-H (2174 nm) (Ma et al., 2017) and to the combination of C-H stretch  
 230 and C-H deformation modes (2390 nm) (Wang et al., 2018).

231 In order to have a more precise idea about the relation between samples and variables  
 232 a PCA model, a non-supervised method was performed with the raw spectra data to  
 233 identify possible sample groupings. The score plot of the two first principal components  
 234 (PCs) is shown in Fig. 2. A total of 98% of the variance is explained by these two first  
 235 PCs (87 and 11% for PC<sub>1</sub> and PC<sub>2</sub>, respectively). Along PC<sub>1</sub>, cocoa shell samples were

236 clearly separated from the remaining ones, in which any clear tendency was observed,  
237 although samples containing high cocoa shell percentages (40% w/w) seemed to be  
238 located closer to the PC<sub>1</sub> values of cocoa shell. According to the X-loading values (data  
239 not shown), the wavelengths with higher discrimination power were 1930, 1420 and 1470  
240 nm for the PC<sub>1</sub> and for the PC<sub>2</sub> were 1644, 1326, 2146, 2310 and 2350 nm. Some of these  
241 peaks (1930, 1470, 2310 and 2350 nm) matched with the main peaks observed in raw  
242 spectra, which have been previously mentioned. The other bands corresponded to the first  
243 overtone of the hydroxyl and amino groups (1420 nm) and first overtone of C-H (1644  
244 nm) (Ribeiro et al., 2011), the second overtone of C-H (1326 nm) (Ma, Wang, Chen,  
245 Cheng, & Lai, 2017) and the combination of C-C and C-H stretching (2146 nm)  
246 (Workman, & Weyer, 2008).

247

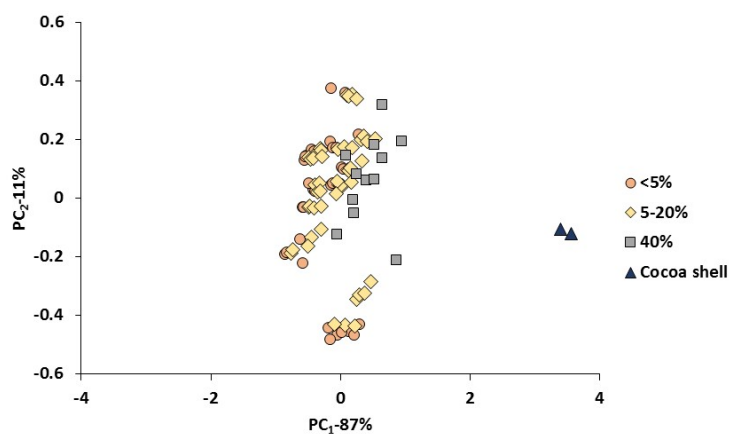


Fig 2. M.A. Quelal-Vásquez et al.

248

249 **Fig 2.** PCA score plot of the two first PCs showing the distribution of all the samples  
250 considered in this study. Samples were labelled as follows: cocoa shell content < 5%,  
251 comprised between 5 and 20%, 40% and pure cocoa shells.

252

253 *3.2. Prediction of the added cocoa shell percentage in cocoa powders by PLS*

254

255 A total of 8 PLS models using all the available wavelengths (700) as variables, one for  
256 each pre-treatment considered in the study, were performed. The results obtained are  
257 summarized in Table 1. At the sight of the results, the best PLS model was the one  
258 constructed using the EMSC+OSC pre-treatment. In order to reduce the high  
259 dimensionality of the spectral data, the most important wavelengths were selected  
260 according to the VIP scores (figure 3). These VIP scores determine the significance of  
261 each variable in the projection used by a given PLS model by means of their coefficients  
262 in every component, jointly with the significance of each component in regression  
263 (Botelho et al., 2015). As it could be observed in Fig. 3, the most important variables are  
264 wavelengths at 1930, 1420 and 1470 nm at positive values of LV<sub>1</sub>, and 2310, 2350 and  
265 1730 nm at negative values of LV<sub>1</sub>. These wavelengths are mostly the same previously  
266 mentioned in both raw and pre-treated spectra, which demonstrated their importance in  
267 cocoa shell content prediction. Most of these wavelengths have been previously described  
268 in literature in the prediction of several compounds (such as fat, carbohydrates,  
269 polysaccharides, moisture, polyphenols, etc.) of cocoa beans and derived products  
270 (Huang et al., 2014; Krähmer et al., 2015; Quelal-Vásconez et al., 2018; Veselá et al.,  
271 2007). Using the EMSC+OSC pre-treatment and the six wavelengths obtained in the VIP  
272 scores as variables, another PLS model was constructed. The results obtained for this  
273 model are also shown in Table 1. Compared to the best model obtained with the same  
274 pre-treatment but using all the available wavelengths, this model is less complex although  
275 all the other parameter values are very similar.

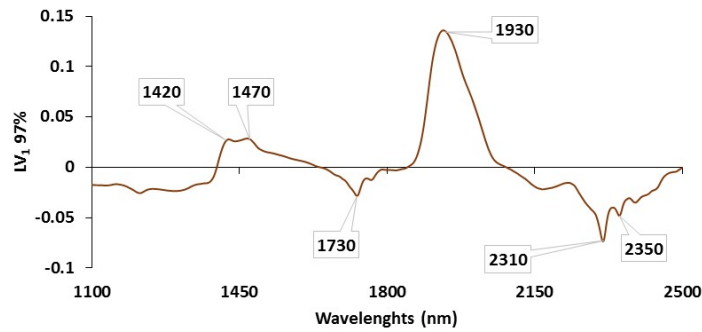


Fig. 3. M.A. Quelal-Vásconez et al.

276

277 **Fig 3.** Variable importance in projection (VIP) scores of the PLS model constructed to  
 278 predict cocoa shell percentages.

279

280 **Table 1**

281 Results of the PLS models constructed for predicting cocoa shell percentage using  
 282 different pre-treatments and different number of wavelengths with a calibration and  
 283 validation sets.

Pretreatment	#W	#LV	Calibration		Cross-validation		Validation			
			R <sup>2</sup> <sub>c</sub>	RMSEC	R <sup>2</sup> <sub>cv</sub>	RMSECV	R <sup>2</sup> <sub>p</sub>	RMSEP	Bias	RPD
Raw data	700	7	0.908	3.68	0.694	6.83	0.930	3.52	0.351	3.46
EMSC	700	7	0.936	3.06	0.857	4.64	0.941	3.24	0.095	3.77
SNV	700	7	0.931	3.18	0.862	4.55	0.940	3.27	0.057	3.72
2nd Der. (S-G)	700	7	0.967	2.20	0.936	3.09	0.955	2.96	-0.021	4.11
OSC	700	1	0.990	1.20	0.989	1.25	0.851	5.16	-0.059	2.36
EMSC-OSC	700	1	0.974	1.92	0.973	2.01	0.967	2.41	0.204	5.06
SNV+OSC	700	1	0.978	1.79	0.976	1.89	0.967	2.55	-0.278	4.77
2nd Der. (S-G)+OSC	700	3	0.944	2.85	0.942	2.96	0.939	3.33	-0.104	3.66
EMSC-OSC	6	1	0.975	1.91	0.973	2.01	0.967	2.43	0.195	5.03

284 #W = number of wavelengths used to construct de model; #LV = latent variables; R<sup>2</sup> =

285 determination coefficient; RMSEC = Root mean square error of calibration; RMSECV =

286 Root mean square error of cross-validation; RMSEP = Root mean square error of

287 prediction; RPD = Ratio prediction deviation; EMSC = Extended multiple scatter  
288 correction; 2<sup>nd</sup> Der. (S-G) = Second derivative and Savitzky Golay smoothing, SNV =  
289 Standard Normal Variate, OSC = Orthogonal signal correction.

290

291

292 The plot representing the predicted versus the measured cocoa shell percentages of the  
293 prediction set samples constructed with PLS data of the model constructed using the 6  
294 wavelengths as variables is shown in Fig. 4. A good linear fit due to the closer relationship  
295 between the reference values and the NIR spectra is observed, displaying the reliability  
296 and accuracy of the NIR in determining the percentage of cocoa shell present in the cocoa  
297 powders.

298

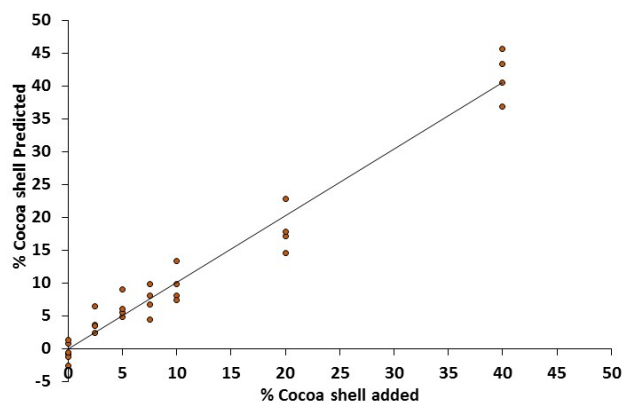


Fig. 4. M.A. Quelal-Vásconez et al.

299

300 **Fig 4.** Predicted versus measured cocoa shell percentages by PLS model constructed  
301 using the 6 main wavelengths in the prediction set.

302

303



304 *3.3. Classification of cocoa powder samples according to the added level of cocoa shell*

305

306 Since PCA is a non-supervised method, and it is not possible to observe a clear  
307 separation between the different sample categories, a supervised discriminant model,  
308 PLS-DA, was next constructed using all the available wavelengths (700) and the EMSC-  
309 OSC pre-treatment. The best model was obtained with 2 LVs with RMSEC and RMSECV  
310 values of 0.24 and 0.28, respectively, with most of the variability explained by the LV<sub>1</sub>  
311 (72%).

312 Next, using the 6 most relevant wavelengths as variables, another PLS-DA model was  
313 constructed. The discriminant plot obtained using the two LVs for the classification of  
314 samples according to the different categories is shown in Fig. 5. As it can be observed in  
315 this figure, separation between the two categories is achieved along LV<sub>1</sub>, with negative  
316 scores related to the samples containing < 5% cocoa shell, and positive scores related to  
317 samples containing 5-40% cocoa shell. Once constructed, the model was validated with  
318 the external validation set samples. The results obtained for both calibration and external  
319 validation sets for this model are included in Table 2. As it can be observed in the  
320 confusion table for the calibration samples, all samples were correctly classified. On the  
321 other hand, for the external validation set, all samples of the <5% category were correctly  
322 classified, while 3 samples of the 5-40% category were misclassified. Even if the number  
323 of misclassified samples is very low, it should be highlighted that all the “misclassified  
324 samples” corresponded to samples containing a 5% cocoa shell (based on fat-free dry  
325 matter), which is the limit established by the Codex Alimentarius, and thus the borderline  
326 of both categories. Next, the PLS-DA classification performance was evaluated by the  
327 sensitivity, specificity and NER values, which are also included in Table 2. Taking into  
328 account the values reported and the comments previously mentioned, it could be

329 concluded that the PLS-DA model constructed is able to reliably discriminate between  
330 samples containing cocoa shell percentages below and upper 5%.

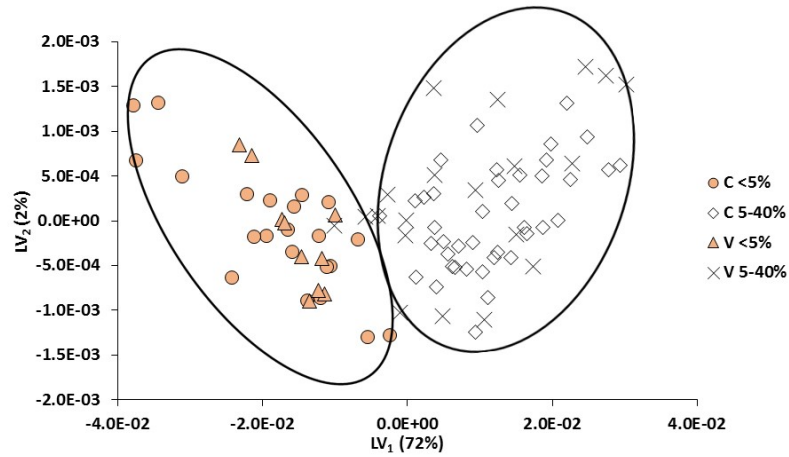


Fig. 5. M.A. Quelal-Vásquez et al.

331  
332 **Fig 5.** PLS-DA discriminant plot constructed using the two first LVs of the model  
333 constructed using the 6 main wavelengths to classify cocoa powders according to the  
334 following categories: cocoa shell content < 5% and cocoa shell content comprised  
335 between 5 and 40%. Both calibration (C<5% and C 5-40%) and external validation  
336 (V<5% and V 5-40%) set samples have been included and represented with different  
337 symbols.

338

339

340

341

342

343

344

345

346 **Table 2**

347

348 Confusion table, sensitivity (SENS), specificity (SPEC) and non-error prediction rates  
 349 (NER) of the PLS-DA model constructed with variable selection to discriminate cocoa  
 350 powders into two categories: cocoa powders with < 5% and between 5-40% cocoa shell.

351

<b>Calibration set samples</b>						
	Category		# Samples	SENS (%)	SPEC (%)	NER (%)
	<5%	5-40%				
<5%	22	0	22	100	100	100
5-40%	0	40	40	100	100	
	22	40	62			

352

<b>External validation set samples</b>						
	Category		# Samples	SENS (%)	SPEC (%)	NER (%)
	<5%	5-40%				
<5%	10	0	10	100	85	92.5
5-40%	3	17	20	85	100	
	13	17	30			

353

354 **4. Conclusions**

355

356 NIR spectroscopy in combination with PLS and PLS-DA statistical models has  
 357 been shown to be a rapid and effective method to determine cocoa shell content in cocoa  
 358 powders. Using a PLS analysis, it was possible to quantify the percentage of cocoa shell  
 359 present in cocoa powders. The best PLS prediction model was constructed using the 6  
 360 main wavelengths (1420, 1470, 1730, 1930, 2310 and 2350) selected according to the  
 361 VIP scores, obtaining 1 LV with  $R^2_C$  and  $R^2_{CV}$  of 0.975 and 0.973, respectively, and  
 362 RMSEC and RMSECV of 1.91 and 2.01, respectively. Regarding the validation samples,  
 363  $R^2_P$  was 0.967 while RMSEP was 2.43, confirming the goodness of the model. On the

364 other hand, the PLS-DA analysis show that 92.5% of the validation set samples were  
365 correctly classified into two groups: samples with a shell content lower than 5%  
366 (considered the acceptance limit in cocoa powders by the Codex Alimentarius) and shell  
367 contents between 5 and 40%. These results indicate that this technology is therefore an  
368 important tool for cocoa producers and clients, who will be able to discriminate among  
369 samples in or out specifications, avoiding the use of destructive techniques that require a  
370 complex preparation of the sample or techniques that imply an important expense for the  
371 company.

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381 **References**

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383 Adnan, A., Hörsten, D. von, Pawelzik, E., & Mörlein, D. (2017). Rapid Prediction of  
384 Moisture Content in Intact Green Coffee Beans Using Near Infrared Spectroscopy.  
385 *Foods*, 6(6), 38.

386 Almeida, M. R., Fidelis, C. H. V, Barata, L. E. S., & Poppi, R. J. (2013). Classification  
387 of Amazonian rosewood essential oil by Raman spectroscopy and PLS-DA with  
388 reliability estimation. *Talanta*, 117, 305–311.

389 Álvarez, C., Pérez, E., Cros, E., Lares, M., Assemat, S., Boulanger, R., & Davrieux, F.  
390 (2012). The use of near infrared spectroscopy to determine the fat, caffeine,  
391 theobromine and (-)-epicatechin contents in unfermented and sun-dried beans of  
392 Criollo cocoa. *Journal of Near Infrared Spectroscopy*, 20(2), 307–315.

393 AOAC, 1984. AOAC, *Official methods of analysis*. Association of Official Analytical  
394 Chemists. 14th ed., Arlington, VA.

395 Berrueta, L. A., Alonso, R. M., & Héberger, K. (2007). Supervised pattern recognition in  
396 food analysis. *Journal of Chromatography A*, 1158(1–2), 196–214.

397 Beckett, S. T. (2009). *Industrial chocolate manufacture and use*. (4<sup>th</sup> ed.). Hoboken, NJ:  
398 Wiley-Blackwell.

399 Botelho, B. G., Reis, N., Oliveira, L. S., & Sena, M. M. (2015). Development and  
400 analytical validation of a screening method for simultaneous detection of five  
401 adulterants in raw milk using mid-infrared spectroscopy and PLS-DA. *Food*  
402 *Chemistry*, 181, 31–37.

403 Bro, R., & Smilde, A. K. (2014). Principal component analysis. *Analytical Methods*, 6(9),  
404 2812–2831.

405 Cantor, S. L., Hoag, S. W., Ellison, C. D., Khan, M. A., & Lyon, R. C. (2011). NIR

406 Spectroscopy Applications in the Development of a Compacted Multiparticulate  
407 System for Modified Release. *AAPS PharmSciTech*, 12(1), 262–278.

408 Codex Alimentarius (2016). Standard for cocoa (cacao) mass (cocoa/chocolate liquor)  
409 and cocoa cake. Codex Stan 141-1983.

410 Dico, G. M. L., Galvano, F., Dugo, G., D'ascenzi, C., Macaluso, A., Vella, A., &  
411 Ferrantelli, V. (2018). Toxic metal levels in cocoa powder and chocolate by ICP-MS  
412 method after microwave-assisted digestion. *Food Chemistry*, 245, 1163–1168.

413 Huang, X. Y., Teye, E., Sam-Amoah, L. K., Han, F. K., Yao, L. Y., & Tchabo, W. (2014).  
414 Rapid measurement of total polyphenols content in cocoa beans by data fusion of  
415 NIR spectroscopy and electronic tongue. *Analytical Methods*, 6(14), 5008–5015.

416 Hug, B., Golay, P. A., Giuffrida, F., Dionisi, F., & Destailats, F. (2006). Development  
417 of a gas–liquid chromatographic method for the analysis of fatty acid tryptamides in  
418 cocoa Products. *Journal of Agricultural and Food Chemistry*, 54(9), 3199–3203.

419 Krähmer, A., Engel, A., Kadow, D., Ali, N., Umaharan, P., Kroh, L. W., & Schulz, H.  
420 (2015). Fast and neat – Determination of biochemical quality parameters in cocoa  
421 using near infrared spectroscopy. *Food Chemistry*, 181, 152–159.

422 Lerma-García, M. J., Cortés, V., Talens, P., & Barat, J. M. (2018). Variety discrimination  
423 of fruits, edible plants, and other foodstuffs and beverages by Infrared Spectroscopy.  
424 In J. Lopes, & C. Sousa (eds.), *Comprehensive Analytical Chemistry volume 80* (pp.  
425 127–163). Oxford: Elsevier.

426 Liu, Y., Sun, X., & Ouyang, A. (2010). Nondestructive measurement of soluble solid  
427 content of navel orange fruit by visible-NIR spectrometric technique with PLSR and  
428 PCA-BPNN. *LWT - Food Science and Technology*, 43(4), 602–607.

429 Ma, H. L., Wang, J. W., Chen, Y. J., & Lai, Z. T. (2017). Rapid authentication of starch  
430 adulterations in ultrafine granular powder of Shanyao by near-infrared spectroscopy

431 coupled with chemometric methods. *Food chemistry*, 215, 108-115.

432 Manfredi, M., Robotti, E., Quasso, F., Mazzucco, E., Calabrese, G., & Marengo, E.  
433 (2018). Fast classification of hazelnut cultivars through portable infrared  
434 spectroscopy and chemometrics. *Spectrochimica Acta - Part A: Molecular and*  
435 *Biomolecular Spectroscopy*, 189, 427–435.

436 Martens, H., Nielsen, J. P., & Engelsen, S. B. (2003). Light scattering and light  
437 absorbance separated by extended multiplicative signal correction. Application to  
438 near-infrared transmission analysis of powder mixtures. *Analytical Chemistry*,  
439 75(3), 394–404.

440 Mendes, F. A. T., & Lima, E. L. (2007). Perfil Agroindustrial do Processamento de  
441 Amêndoas de Cacau em Pequena Escala no Estado do Pará. SEBRAE/PA.

442 Osborne, B. G., Fearn, T., & Hindle, P. H. (1993). *Practical NIR spectroscopy with*  
443 *applications in food and beverage analysis*. Longman Scientific & Technical, Wiley,  
444 Harlow, Essex, England, New York.

445 Okiyama, D. C., Navarro, S. L., & Rodrigues, C. E. (2017). Cocoa shell and its  
446 compounds: Applications in the food industry. *Trends in Food Science &*  
447 *Technology*, 63, 103–112.

448 Prats-Montalbán, J. M., Jerez-Rozo, J. I., Románach, R. J., & Ferrer, A. (2012). MIA and  
449 NIR Chemical Imaging for pharmaceutical product characterization. *Chemometrics*  
450 *and Intelligent Laboratory Systems*, 117, 240–249.

451 Teye, E., Huang, X., Dai, H., & Chen, Q. (2013). Rapid differentiation of Ghana cocoa  
452 beans by FT-NIR spectroscopy coupled with multivariate classification.  
453 *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 114, 183–  
454 189.

455 Quelal-Vásquez, M. A., Pérez-Esteve, É., Arnau-Bonachera, A., Barat, J. M., & Talens,

456 P. (2018). Rapid fraud detection of cocoa powder with carob flour using near  
457 infrared spectroscopy. *Food Control*, 92, 183–189.

458 Ribeiro, J. S., Ferreira, M. M. C., & Salva, T. J. G. (2011). Chemometric models for the  
459 quantitative descriptive sensory analysis of Arabica coffee beverages using near  
460 infrared spectroscopy. *Talanta*, 83(5), 1352–1358.

461 Veselá, A., Barros, A. S., Synytsya, A., Delgadillo, I., Čopíková, J., & Coimbra, M. A.  
462 (2007). Infrared spectroscopy and outer product analysis for quantification of fat,  
463 nitrogen, and moisture of cocoa powder. *Analytica Chimica Acta*, 601(1), 77–86.

464 Wang, J., Zhang, X., Sun, S., Sun, X., Li, Q., & Zhang, Z. (2018). Online determination  
465 of quality parameters of dried soybean protein–lipid films (Fuzhu) by NIR  
466 spectroscopy combined with chemometrics. *Journal of Food Measurement and*  
467 *Characterization*, <https://doi.org/10.1007/s11694-018-9762-z>.

468 Workman Jr, J., & Weyer, L. (2007). *Practical guide to interpretive near-infrared*  
469 *spectroscopy*. Taylor and Francis Group, Boca Raton, FL: CRC Press.

470 Yang, W., Hu, M., Chen, S., Wang, Q., Zhu, S., Dai, J., & Li, X. (2015). Identification of  
471 Adulterated Cocoa Powder Using Chromatographic Fingerprints of Polysaccharides  
472 Coupled with Principal Component Analysis. *Food Analytical Methods*, 8(9), 2360–  
473 2367.

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