Non-destructive determination of fatty acid composition of in-shell and shelled almonds using handheld NIRS sensors

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Abstract

One of the major compounds in almond kernels, which determines their nutritional quality, are lipids. The aim of this research was to determine the fatty acid profile in intact in-shell and shelled almonds (145 samples) using two new generation handheld near infrared spectroscopy (NIRS) sensors, of different optical design and technical specifications, adapted for *in situ* analysis in different stages in the food supply chain: in the industry after harvest, at the reception points and during postharvest storage. For both instruments, two procedures for taking near infrared (NIR) spectra were tested: (1) static, where point spectral readings were taken of almonds placed on trays; (2) dynamic, where spectra were taken by scanning the entire trays. Modified partial least squares (MPLS) regression models were developed using NIR spectra with different combinations of signal pre-treatments — derivative and scatter correction methods. The residual predictive deviation for cross validation (RPD_{cv}) of the best models developed for the prediction of palmitic, stearic, oleic, and linoleic acids using shelled almonds were 2.40, 2.16, 3.98, and 3.77, respectively, and 1.73, 1.73, 2.02, and 2.11 for the in-shell almonds. These results confirm the feasibility of NIRS technology to measure the fatty acid profile in in-shell and shelled almonds. A comparison between the presentation mode (in-shell or shelled) and analysis mode (static or dynamic) showed that the best results were obtained for shelled almonds analysed in dynamic mode.

Keywords: almonds, *in situ* analysis, NIRS sensors, fatty acid profile, quality, shelf life determination.

1. Introduction

Almonds (*Prunus dulcis* Miller) are characterized by their high content in lipids, which are of great nutritional value, constituting 60 % of the total kernel mass (Senesi et al., 1996). These mainly consist of unsaturated fatty acids – oleic and linoleic acids, which together generally account for more than 90 % of the fatty acids (Fernández-Cuesta et al., 2013). The consumption of these unsaturated fatty acids available in the almond kernels can have potential benefits for human health, lowering the level of triglycerides and increasing high-density lipoprotein cholesterol (Hyson et al., 2002). In addition, the consumption of these oleic and linoleic acids has been linked to a reduction in the incidence of diabetes and cardiovascular diseases (Richardson et al., 2009; Virtanen et al., 2014; Roncero et al., 2016; Becerra-Tomás et al., 2019). Oleic acid is the prevailing constituent of monounsaturated lipids in almonds and contributes to its oxidative stability (Venkatachalam and Sathe, 2006). The polyunsaturated fatty acids in almonds not only give them their nutritional value, but also make them more prone to autoxidation, which accelerates their deterioration and reduces shelf life. Thus, high levels of linoleic acid could indicate almond spoilage (Ros and Mataix, 2006; Kodad and Socias i Company, 2008; Martínez et al., 2013; Oliveira et al., 2019). For this reason, one of the most important quality indexes is the oleic/linoleic acid ratio; high values of this ratio provide stability in oils and better nutritional value (Kodad et al., 2013).

The composition, quality and shelf life of almonds are influenced by many factors such as the cultivar, agronomic practices and the environmental conditions during the growing season (Gama et al., 2018). Different authors have shown that the quality of the kernels, mainly evident in the fatty acid composition, oil content, protein content, rate of rancidity, oxidative stability, peroxide formation and shelf life, are all influenced by the

67 almond cultivar (Abdallah et al., 1998; Severini et al., 2000; Sathe et al., 2008; Kodad et
68 al., 2013; Maestri et al., 2015; Yildirim et al., 2016). Others have also reported that the
69 alpha-tocopherol content, squalene concentration, sugar content and sugar composition
70 of almonds are also heavily influenced by the cultivar (Nanos et al., 2002; Maestri et al.,
71 2015). The kernel quality and the fatty acid composition of almonds may also be affected
72 by the stage of maturity and time of harvest. Thus, late-harvested almonds have a higher
73 kernel dry mass, more sugar, lower oleic acid, higher linoleic acid and lower oil quality
74 than early-harvested almonds (Nanos et al., 2002; Kazantzis et al., 2003; Piscopo et al.,
75 2010). However, almonds harvested too early in the season can have immature kernels
76 that have not fully converted their carbohydrates into oil (Nanos et al., 2002). In addition
77 to pre-harvest factors, the type of storage (in-shell or shelled) can also influence the
78 quality and shelf life of the almonds (Kazantzis et al., 2003). Hardenburg et al. (1986)
79 reported stored shelled almonds to be more sensitive to lipid oxidation than in-shell
80 almonds, with a shelf life of in-shell almonds up to twice as long as those stored without
81 shell. As a result, fatty acid composition can be used as a measure of the quality and shelf
82 life of this tree nut and, in turn, to measure the commercial and industrial use of the
83 almond kernels (Zacheo et al., 1998; Koyuncu et al., 2005; Socias i Company et al., 2008;
84 Bai et al., 2017).

85 Currently, the fatty acid profile is generally determined in almonds using solvent
86 extraction techniques. However, solvent extraction has several drawbacks, including high
87 capital equipment cost, operational expenditure and concerns for environmental pollution
88 (Fernández-Cuesta et al., 2013).

89 In recent years, non-destructive techniques such as NIRS has been successfully
90 used to predict oil content and fatty acid profile in almonds (Fernández-Cuesta et al.,
91 2013). These measurements were taken in the fruit after grinding (almond flour), using a

92 monochromator, which is only suitable for analysing the product in the laboratory. No
93 previous studies aiming at the prediction of the fatty acid profile of intact in-shell or
94 shelled almonds using portable or online NIRS instruments have been found in the
95 literature. However, currently, there is a clear need for a fast and efficient way of
96 measuring the fatty composition of the almonds *in situ* in a non-destructive way, when
97 the almonds are received by the industry and during the postharvest storage. Nowadays,
98 there is a wide range of different portable instruments available, which clearly shows how
99 the use of NIRS technology has evolved and which allow these analyses to be carried out
100 effectively *in situ*. However, the wide diversity in the characteristics and features of these
101 portable NIRS sensors means that they need to be evaluated in advance to determine
102 which is the most suitable sensor for a particular product or parameter.

103 The objective of this research was to measure the fatty acid profile in intact in-
104 shell and shelled almonds using two new generation handheld NIRS sensors of different
105 optical design and technical specifications, suitable for the *in situ* analysis of almonds
106 when they are received by the industry, in order to establish the nutritional quality of the
107 product at that moment.

108

109 **2. Materials and methods**

110

111 *2.1. Sampling*

112

113 The plant material consisted of 84 samples of in-shell sweet almonds (*Prunus*
114 *dulcis* Mill., cv. 'Antoñeta', 'Belona', 'Guara', 'Lauranne', 'Soleta', and 'Vairon') and 84
115 samples of shelled sweet almonds, of the same varieties and batches as above. In addition,
116 there were 61 samples of bitter almonds of non-specific varieties, initially in-shell, which

117 were then shelled manually, making a total of 145 samples of in-shell and shelled almonds
118 analysed. The samples weighed approximately 1 kg each and were collected during the
119 2018-2019 harvesting season in the different provinces of the region of Andalusia
120 (Spain). On arrival at the laboratory, the almonds were immediately placed in dark,
121 refrigerated storage at 4 ºC and 65 % relative humidity until the following day, when
122 laboratory testing was performed. Prior to measurement, each sample was left to stabilize
123 at the laboratory temperature of 20 ºC.

124

125 *2.2. Instrumentation and NIR spectra acquisition*

126

127 The NIR spectra of the in-shell and shelled sweet and bitter almonds were taken
128 using two portable handheld NIRS instruments of different optical designs, both suitable
129 for the *in situ* analysis of the product (Table 1).

130 Initially, a compact, handheld instrument based on diode-array technology
131 (Aurora spectrophotometer, GraiNit S.r.l., Padova, Italia) was used. The
132 spectrophotometer works in reflectance mode in the spectral range 950-1650 nm, taking
133 data every 2 nm, with an optical window of 1256 mm^2. The sensor integration time was
134 6.57 ms and each spectrum was the mean of 50 scans. This instrument has an internal
135 reference, which facilitates easy calibration. Acquisition of the spectra was carried out by
136 means of the UCal 4™ software (Unity Scientific LLC, Milford, MA, USA). Each sample
137 of in-shell and shelled almonds was uniformly distributed on a white plastic tray covering
138 the whole surface, and two modes of analysis were tested: static and dynamic. For the
139 analysis in static mode, the sensor was placed at 4 different points in the tray and the
140 equipment was kept still in contact with the almonds while the spectrum was recorded.
141 Thus, a total of 4 measurements were taken per sample. In the dynamic mode, the 4

142 spectra taken per sample were obtained by moving the sensor in contact with the almonds
143 along the tray, at a speed that enabled us to cover the entire area of the tray. In both cases,
144 the 4 spectra were averaged to provide a mean spectrum per sample in the two analysis
145 modes.

146 Spectra of the almonds were also taken using a spectrophotometer that
147 incorporates the Linear Variable Filters (LVF) technology as the dispersion element
148 (MicroNIR™ Pro 1700, VIAVI Solutions, Inc., San Jose, California, USA), which works
149 in reflectance mode in the spectral range 908 to 1676 nm with a constant interval of 6.2
150 nm. This portable miniature spectrophotometer is extremely light (64 g, not including the
151 150 g handle and the acquisition and data processing device), with an optical window of
152 around 227 mm^2. The sensor integration time was set at 11 ms and each spectrum was the
153 mean of 200 scans. Spectra acquisition was carried out using the VIAVI MicroNIR
154 software Pro version 2.2 (VIAVI Solutions, Inc., San Jose, California, USA). The
155 instrument's performance was checked every 10 min. A white reference measurement
156 was obtained using a NIR reflectance standard (Spectralon™) with 99 % diffuse
157 reflectance, while a dark reference was obtained from a fixed point on the floor of the
158 room. For in-shell and shelled almonds, the analyses in static and dynamic modes were
159 also carried out following the same procedure described above, with the only difference
160 that in the static mode, six spectra were taken per sample, due to the smaller spectral
161 window of this instrument. Next, the spectra were averaged to obtain a mean spectrum
162 per sample for each presentation form (in-shell and shelled almonds) and analysis mode
163 (static and dynamic).

164

165 *2.3. Reference data*

166

167 The fatty acid (FA) profile was measured by capillary gas chromatography of the
168 fatty acid methyl esters (FAMEs). These FAMEs were prepared by trans-esterification
169 with KOH according to the official Commission Regulation (EEC) No 2568/91, Annex
170 X (OJEC, 1991). The FAMEs were separated using a gas chromatograph Alignent 7890A
171 (Agilent Technologies, Inc., Santa Clara, CA, USA) and afterwards detected using a
172 flame ionization detector (FID), equipped with a capillary column (RESTEK Rtx2320 60
173 m × 0.25 mm i.d.) and 0.20 µm film thickness (RESTEK, Bellefonte, PA, USA). The
174 carrier gas was helium and the flow rate was 1 mL min^{-1}. The temperatures of the injector
175 and detector were maintained at 250 ºC and 260 ºC, respectively. The initial column
176 temperature was 175 ºC for 19 min. The oven temperature was gradually increased from
177 175 ºC to 200 ºC at 5 ºC min^{-1} ramp rate, and it was maintained at 200 ºC for 15 min.
178 Injection volume was 1.0 µL. FAME identification was based on retention times as
179 compared with those of the standard FAME mixture (Sigma-Aldrich, Madrid, Spain). For
180 calibration development, only the four main fatty acids in almond composition were used,
181 i.e. palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids. The
182 relative concentration of each acid was expressed as the percentage of total fatty acids.
183
184 *2.4. Data processing*
185
186 Data pre-processing and chemometric treatments were performed using the
187 WinISI II software package version 1.50 (Infrasoft International LLC, Port Matilda, PA,
188 USA) (ISI, 2000).
189
190 *2.4.1. Spectral repeatability*

191 Different procedures were evaluated to determine the spectrum quality for both
192 instruments using the root mean square (RMS) statistic, which is defined as the averaged
193 root mean square of differences between the different subsamples scanned at the *n*
194 wavelengths (Shenk and Westerhaus, 1995b, 1996). This statistic indicates the similarity
195 between different spectra of a single sample. Initially, four spectra were taken at the same
196 point of the sample for the two sample presentations studied (in-shell and shelled
197 almonds). A total of 10 samples of in-shell and shelled almonds were used. Next, 10
198 samples of in-shell and shelled almonds were analysed by taking four spectra in static and
199 dynamic modes using the diode-array spectrophotometer and six and four spectra in static
200 and dynamic modes, respectively, using the LVF instrument, following the procedure for
201 obtaining spectral information explained in Section 2.2. An admissible limit for spectrum
202 quality and repeatability was set following the procedure described by Martínez et al.
203 (1998) to calculate the standard deviation limit (STD_{limit}) from the RMS statistic and
204 obtain an RMS cut-off value. The SDT_{limit} values for the different analysis modes, sample
205 presentations and instruments were compared for the two alternatives using Fisher's F
206 test (Mark and Workman, 2003). F critical was calculated considering 9 degrees of
207 freedom and $P = 0.05$.

208

209 *2.4.2. Quantitative models: definition of sets, calibration and validation procedures*

210 Initially, the structure and spectral variability of the population was studied to
211 select the calibration and internal validation sets for this study. To achieve this, the
212 CENTER algorithm was used. This algorithm finds the centre of the spectral population
213 and calculates the Mahalanobis distance (GH) between each sample and the centre of the
214 population, expressed in principal components (Shenk and Westerhaus, 1995a). The
215 CENTER algorithm was applied separately for each of the eight available groups (in-shell

216 and shelled almonds analysed in static and dynamic modes with both instruments). A
217 combination of mathematical pre-treatments was applied — standard normal variate
218 (SNV) and de-trending (DT) for scatter correction (Barnes et al., 1989), together with the
219 1,5,5,1 derivative treatment, where the first digit is the number of the derivative, the
220 second the gap over which the derivative is calculated, the third the number of data points
221 in a running average or smoothing, and the fourth the second smoothing (Shenk and
222 Westerhaus, 1995b). Those samples with high GH values (GH > 3.5) were identified as
223 anomalous or spectral outliers, for the different presentation forms and analysis modes.
224 The study of outliers was carried out together, excluding the same samples in all the
225 groups if their removal was justified. Having ordered the sample set by spectral distances
226 and, once the spectral outliers were removed from all the sets, the structured selection of
227 the calibration and internal validation sets was carried out following the procedure
228 outlined by Shenk and Westerhaus (1991). To achieve this, in order to match the
229 calibration and internal validation sets with the same samples and, consequently, to be
230 able to compare the prediction accuracy of the quantitative models, 1 out of every 4
231 samples was selected from the set of shelled almonds analysed in dynamic mode using
232 the LVF spectrophotometer to build the internal validation set, while the remaining
233 samples were used to build the calibration set. Likewise, the same samples were selected
234 from the other seven groups to constitute their respective calibration and internal
235 validation sample sets.

236 The prediction of the main four fatty acids (palmitic, stearic, oleic, and linoleic)
237 in in-shell and shelled almonds analysed in static and dynamic modes was devised using
238 MPLS regression with five cross validation groups (Shenk and Westerhaus, 1995a). The
239 full spectral range of the instruments was used. For each of the four fatty acids analysed,
240 two derivative treatments (1,5,5,1 and 2,5,5,1), in combination with SNV and DT for

241 scatter correction, were tested. The best models were selected by assessing their
242 performance using the coefficient of determination for cross validation (R^2_{cv}), the
243 standard error of cross validation (SECV), and the residual predictive deviation for cross
244 validation (RPD_{cv}), calculated as the ratio of the standard deviation (SD) of the reference
245 data for calibration to the SECV. In addition, the regression coefficients for the best
246 calibration models for the four fatty acids in shelled almonds analysed in dynamic mode
247 using the diode-array spectrophotometer were also assessed in order to identify those
248 wavelengths contributing most to the prediction of the parameters evaluated (Martens and
249 Naes, 1989).

250 To identify the most suitable spectrophotometer, analysis mode (static or
251 dynamic), and sample presentation (in-shell or shelled) for the *in situ* prediction of the
252 fatty acid composition in almonds, tests were run to identify potential differences between
253 the models developed. To that end, the SECV values for the best models previously
254 selected for the four fatty acids studied were compared using Fisher's F test (Mark and
255 Workman, 2003), with $P = 0.05$. Those models obtained using the most suitable analysis
256 mode and sample presentation for the prediction of the four fatty acids in the in-shell and
257 shelled almonds analysed with both instruments were selected using the statistical criteria
258 mentioned above, and later subjected to internal validation following the protocol
259 outlined by Windham et al. (1989) and the internal validation sets previously selected.

260

261 **3. Results and discussion**

262

263 *3.1. Spectral analysis of the almonds and spectral repeatability*

264

265 The raw spectra and mean second derivative absorbance spectra of the in-shell
266 and shelled almonds analysed in dynamic mode using both instruments, together with the
267 most relevant absorption bands (Fig. 1) showed, in the NIR region, a peak at around 1150
268 nm, which corresponds to the C-H bonds of aromatic compounds, a peak at around 1200
269 nm that corresponds to the presence of lipids, and a peak at 1410 nm related to the first
270 overtone absorbance of the O-H functional groups (Shenk et al., 2008).

271 Studying the quality and repeatability of the collected spectra is a key step in the
272 attempt to build accurate, robust models for the characterization of almonds. For the first
273 procedure tested (analysing 10 in-shell and shelled almond samples and taking four
274 spectra of each of them at the same point), the STD_{limit} values obtained for in-shell and
275 shelled almonds when the diode-array instrument was used were 23,300 µlog (1/R) and
276 23,072 µlog (1/R), respectively. No significant differences were found between these
277 values ($P > 0.05$). Likewise, when the analysis was carried out using the LVF instrument,
278 the STD_{limit} values obtained for in-shell and shelled almonds were 23,839 µlog (1/R) and
279 24,721 µlog (1/R), respectively, which were proved not to be significantly different ($P >$
280 0.05). This indicates that the repeatability of the instruments was not affected by the type
281 of sample analysed when the spectra were acquired at the same point of the sample. In
282 addition, no significant differences ($P > 0.05$) were found when the results obtained for
283 both instruments for in-shell and shelled almonds were compared.

284 Likewise, we studied the influence in the spectral quality of the analysis mode
285 (static or dynamic) in the in-shell and shelled product analysed with both instruments on
286 the STD_{limit} statistic. In all cases, the STD_{limit} value was significantly higher ($P < 0.05$)
287 for those samples analysed in static mode (Table 2). This was to be expected, since a
288 greater quantity of the sample can be analysed in dynamic mode, and the potential
289 differences between kernels of the same sample would be minimised after averaging the

290 50 or 200 scans taken by the diode-array and LFV spectrophotometers per sample
291 analysed, respectively. Next, the sample presentation form was assessed, considering only
292 the dynamic mode of analysis. The STD_{limit} values obtained using the diode-array
293 instrument were shown to be significantly lower ($P < 0.05$) for those samples analysed in
294 the shelled almonds compared to those analysed in-shell. The light interaction with the
295 almond shell could favour the scatter effect, by which the part of the light which falls on
296 the sample cannot be collected by the detectors and is lost. Additive and multiplicative
297 effects can be observed in the spectra of the samples analysed in-shell (Fig. 1A), with
298 these samples showing higher absorbance values. These effects could partially account
299 for the difference between the STD_{limit} statistical values of the in-shell and shelled
300 samples, since the occurrence of more or less light scattering when analysing the sub-
301 samples of in-shell almonds could lead to greater differences and, therefore, less
302 repeatability. Furthermore, the shape of the in-shell almonds made the surface of the
303 samples on which the NIRS analysis was carried out less homogeneous than in the case
304 of the shelled almonds, making it more difficult to analyse the in-shell almonds. Similar
305 comments can be made regarding the spectra taken with the LVF device (Fig. 1B),
306 although in this case the differences found were not significant ($P > 0.05$). Furthermore,
307 a comparison between the two instruments used in this study was carried out. To perform
308 this comparison, the best analysis mode and sample presentation form in terms of $STD_{límit}$
309 (shelled product and dynamic mode) were considered. The diode-array instrument
310 presented significantly lower values ($P < 0.05$) for STD_{limit} compared to the LVF
311 instrument, which may reflect differences in the measuring area (1256 mm^2 *versus* 227
312 mm^2). This is because the diode-array instrument has a larger window size and detects a
313 greater variability in each spectrum taken, which may cause the differences between the
314 different spectra of the same sample to be smaller.

315 The results obtained showed the importance of considering the sample variability
316 when a STD limit for collected spectra has to be established. Consequently, in this study,
317 the STD limits were chosen when the spectra were taken at different points (static mode)
318 of the sample or moving the sensor in contact with the almonds along the tray (dynamic
319 mode), since when the spectra were taken at the same point of the sample, only the
320 repeatability of the instrument itself was being evaluated, rather than the influence of the
321 heterogeneity of the sample. In addition, it must be noted that the influence of the
322 heterogeneity of the sample could be minimised when the analysis is carried out in
323 dynamic mode.
324
325 *3.2. Population characterization*
326
327 Prior to the selection of samples to build the calibration and validation groups, we
328 identified a total of four outliers belonging to the four groups of in-shell samples analysed
329 (GH > 3.5). A detailed study of the spectral outliers showed that these samples presented
330 extreme reference values for palmitic, oleic and linoleic acids. These four outliers were
331 removed from the eight sets of almonds.
332 The selection protocol outlined by Shenk and Westerhaus (1991) proved suitable,
333 in that the calibration and validation sets displayed similar values for range, mean and SD
334 for all the studied parameters. Additionally, the validation set ranges lay within those of
335 the calibration set (Table 3). Those parameters with the greatest variability were stearic
336 ($CV_{calibration}$ = 20.28 %; $CV_{validation}$ = 20.93 %), and linoleic acids ($CV_{calibration}$ = 18.07 %;
337 $CV_{validation}$ = 14.04 %), whereas palmitic ($CV_{calibration}$ = 9.36 %; $CV_{validation}$ = 7.95 %) and
338 oleic ($CV_{calibration}$ = 6.33 %; $CV_{validation}$ = 4.86 %) acids showed a lower variability. These
339 differences in variability could be associated to the almond cultivars analysed in this

340 study. These results are in line with those reported by Zamany et al. (2017) in a study on
341 the fat composition of 20 almond cultivars, who indicated the existence of significant
342 differences ($P < 0.05$) between the percentages of palmitic, stearic, oleic and linoleic acids
343 in these cultivars, and that the stearic and linoleic acids showed the greatest variability.
344
345 *3.3. Development of models and cross validation results for the prediction of the fatty*
346 *acid composition in almonds using MPLS regression*
347
348 The results for the prediction of the saturated fatty acid (SFA) content (Table 4),
349 showed, as regards palmitic acid content, that the models developed using in-shell
350 almonds analysed with the diode-array instrument in static and dynamic modes would
351 enable us to discriminate between high, medium and low values of the parameter tested,
352 whereas for the LVF instrument, the models for in-shell almonds in both modes of
353 analysis would allow us to distinguish between low and high values of this acid (Shenk
354 and Westerhaus, 1996; Williams, 2001). Additionally, the predictive capacity of the four
355 models (product analysed in static and dynamic modes with both instruments) developed
356 for the prediction of this fatty acid in shelled almonds could be considered as good (Shenk
357 and Westerhaus, 1996; Williams, 2001), which means that these results would allow us
358 to determine the almonds' palmitic acid content with a similar precision to that obtained
359 using the traditional reference methods, and consequently, our models would meet the
360 quality standards established by the industry. All the models devised for the prediction of
361 stearic acid in in-shell almonds using the two instruments tested showed a predictive
362 capacity which allowed the samples to be classified as high, medium and low values.
363 Besides that, models with good predictive capacity were obtained for shelled almonds
364 analysed in dynamic mode, whereas for the static mode, the models allowed us to classify

365 the product into high, medium and low content of stearic acid, also using both instruments
366 (Shenk and Westerhaus, 1996; Williams, 2001). Nicolaï et al. (2007) indicated that RPD_{cv}
367 values between 1.5 and 2 could discriminate between low and high values of the predicted
368 variable, while a value between 2 and 2.5 indicates that coarse quantitative predictions
369 are possible. Measuring these SFAs is of key importance, since they regulate the oxidation
370 and deterioration of the almond postharvest, with those almonds with high levels of SFAs
371 being less susceptible to lipid oxidation and accelerated deterioration (Pleasance et al.,
372 2018). However, SFAs can have negative effects on the human cardiovascular system, as
373 they increase low-density lipoprotein cholesterol (LDL-c) (Zock, 2006; Kodad and Socias
374 i Company, 2008), which makes it necessary to quantify these SFAs in food products.
375 Nevertheless, in the almonds analysed in this study, palmitic acid represented 6.52 ± 0.59
376 % and stearic acid 2.11 ± 0.45 %.

377 The results for the prediction of the monounsaturated (oleic acid) fatty acid
378 (MUFA) and polyunsaturated (linoleic acid) fatty acid (PUFA) content (Table 5) showed,
379 with regard to oleic acid, that the predictive capacity of the models developed for in-shell
380 almonds with both instruments in dynamic mode was considered as good. Additionally,
381 the results obtained for the prediction of this acid in shelled almonds using the diode-
382 array instrument in dynamic mode showed an excellent predictive capacity, according to
383 Shenk and Westerhaus (1996) and Williams (2001). For linoleic acid, the models
384 developed using in-shell almonds analysed with the diode-array instrument in dynamic
385 mode and those performed with the LVF instrument, in both static and dynamic modes,
386 had a predictive capacity which can be considered as good when interpreting the
387 coefficient of determination of cross validation, as proposed by Shenk and Westerhaus
388 (1996) and Williams (2001). Likewise, the predictive models for shelled almonds

389 analysed with the diode-array instrument in dynamic mode showed an excellent
390 predictive capacity (Shenk and Westerhaus, 1996; Williams, 2001).

391 Therefore, the models developed in this study are of great interest to the industry
392 since they would enable us to estimate the shelf life of almonds and their quality
393 throughout the postharvest period, serving as a means of supporting decision-making
394 when managing batches of the product.

395 The comparisons carried out to identify the best sample presentation form,
396 analysis mode and instrument used (Table 4 and Table 5) showed that the sample
397 presentation form actually influenced the predictive capacity of the models. The SECV
398 values obtained were shown to be equal or significantly lower ($P < 0.05$), for all the
399 models developed using spectra from shelled almonds, than the values for in-shell
400 almonds, as could be expected. Nevertheless, promising results were obtained for the *in*
401 *situ* analysis of almond samples when they are received in the industry before they are
402 shelled and processed. This is of major importance, since prior to any industrial
403 processing, NIRS could provide information about the quality of the almonds received.
404 The results obtained using the dynamic mode of analysis produced, in all cases, equal or
405 significantly better results ($P < 0.05$) compared to the static mode of analysis. The reason
406 for this could be the greater amount of the sample analysed in dynamic mode, so that a
407 more representative spectrum was obtained from the sample under analysis. The results
408 also showed (Table 4 and Table 5) that although the predictive capacity of both
409 instruments used was fairly similar for most of the models obtained, significant
410 differences ($P < 0.05$) were found for the prediction of palmitic acid in in-shell almonds
411 in dynamic mode and for the prediction of oleic and linoleic acids in shelled almonds in
412 dynamic mode, with the diode-array instrument showing a better prediction capacity. As
413 explained in previous sections, this could be due to the differences in the area of the

414 optical windows of the instruments tested, as well as to the fact that the measurement
415 interval along the spectral range is lower in the diode-array (2 nm) than in the LVF (6.2
416 nm).
417 The contributions of individual wavelengths to the prediction of the main four
418 fatty acids in shelled almonds analysed using the diode-array instrument in dynamic mode
419 (Fig. 2) showed that the regions around 1160–1200 nm and 1500–1600 nm, which can be
420 related to the second overtone of C–H bonds and first overtone and combination bands of
421 the –OH group, respectively (Shenk et al., 2008; Prades et al., 2012), were of considerable
422 importance.
423
424 *3.4. Internal validation*
425 The calibration models obtained using both spectrophotometers and the best
426 analysis mode (dynamic) were subjected to an internal validation procedure.
427 Although the best calibration models were obtained for the shelled almonds,
428 owing to the importance of the screening of the almonds quality at the reception points in
429 the processing industries, the best models obtained for the prediction of the fatty acid
430 profile in in-shell almonds using both instruments were also internally validated (Fig. 3
431 and Fig. 4). According to Nicolaï et al. (2007), based on the residual predictive deviation
432 for prediction (RPD_p) values calculated as the ratio of the SD to the standard error of
433 prediction (SEP), the models developed for the prediction of stearic acid in in-shell
434 almonds using the diode-array instrument could discriminate between low and high
435 values of this FA, while for palmitic acid using both instruments and for oleic and linoleic
436 acids when the LVF instrument was used, the capacity of the developed models was low.
437 The results obtained for the prediction of stearic acid using the LVF instrument and for
438 the models developed for the prediction of oleic and linoleic acids using the diode-array

439 instrument showed that coarse quantitative predictions were possible for these acids.
440 Following the protocol of Windham et al. (1989), the slope values for the four FAs when
441 the models were devised with the diode-array instrument and for the palmitic, oleic and
442 linoleic acids when the LVF instrument was used, did not fall within the recommended
443 interval values, despite being close. As a result, these equations can be taken as a first
444 step in the *in situ* measurement of the quality of almonds when they are received in the
445 industry using a handheld instrument, since all the models met with the other validation
446 requirements, $R^2{}_p > 0.6$ (except the ones devised for palmitic acid with both instruments
447 and the one developed for oleic acid using the LVF instrument), and SEP(c) and bias lay
448 within the confidence limits. Furthermore, the equation developed to predict stearic acid
449 content using the LVF instrument could be applied routinely, since it met all the validation
450 requirements established by Windham et al. (1989). Although no previous studies for the
451 prediction of the fatty acid profile in intact in-shell and shelled almonds have been found
452 in the literature, similar studies involving other products such as other in-shell nuts can
453 be highlighted. Sundaram et al. (2010) measured the fatty acid composition of Valencia-
454 type in-shell peanuts using a monochromator working on reflectance in a spectral range
455 of 400-2500 nm, and reported better results for palmitic ($RPD_p = 2.85$; SEP = 1.56 %),
456 stearic ($RPD_p = 3.02$; SEP = 0.53 %), oleic ($RPD_p = 3.72$; SEP = 4.48 %), and linoleic
457 ($RPD_p = 2.30$; SEP = 7.76 %) acids than those obtained in this study. However, in addition
458 to using a NIRS laboratory instrument with different benefits from those tested here, it is
459 important to take into account other factors such as the type of fruit analysed and the type
460 of shell of each product, since peanuts have a thinner shell than almonds.

461 Internal validation statistics for the best models for the four fatty acids tested, for
462 shelled almonds analysed in dynamic mode using both instruments (Fig. 5 and Fig. 6),
463 showed that the models developed for the prediction of palmitic and stearic acids with

464 both instruments met the validation requirements established by Windham et al. (1989)
465 in terms of R^2_p ($R^2_p > 0.6$) and slope ($1.10 > \text{slope} > 0.90$), while SEP(c) and bias lay
466 within the confidence limits. Consequently, these equations could guarantee accurate
467 prediction and could be applied in routine analysis. However, the slope values of the
468 models devised with both instruments for the prediction of oleic and linoleic acids did not
469 meet the validation requirements established by these authors, despite being extremely
470 close. Therefore, these models could be considered as a first step in the *in situ*
471 measurement of the oleic and linoleic content in intact shelled almonds. The similarity in
472 the SEP and SECV values confirms that the latter statistic is a good estimator of the SEP
473 (Shenk et al., 1989). The RPD_p values obtained for the four fatty acids when the LVF
474 instrument was used indicate that coarse quantitative predictions are possible. When the
475 models were devised using the diode-array spectrophotometer, the RPD_p values obtained
476 for stearic and linoleic acids correspond to a good prediction accuracy of the models,
477 while the RPD_p values for palmitic and oleic acids indicate that an excellent prediction
478 accuracy could be obtained using those models (Nicolaï et al., 2007). In a study conducted
479 by Fernández-Cuesta et al. (2013), a monochromator working in reflectance in the
480 spectral range of 400-2500 nm was used to measure the palmitic (RPD_p = 1.41; SEP =
481 0.34 %) and stearic (RPD_p = 1.44; SEP = 0.34 %) acids content in ground almonds. The
482 results reported by the authors were inferior to the ones obtained here for the prediction
483 of these SFAs in intact shelled almonds, despite the fact that they analysed ground
484 samples, while in the present study intact samples were analysed, making the application
485 developed here more challenging. In addition, the results of this research were an
486 improvement on those reported by Fox and Cruickshank (2005), who measured the oleic
487 (RPD_p = 2.81; SEP = 6.4 %) acid content in shelled peanuts using a monochromator
488 working on reflectance in the spectral range of 400-2500 nm. Davrieux et al. (2010)

developed NIRS models for the prediction of fatty acids in ground shea tree nuts. The results here obtained surpass those reported by these authors for palmitic (RPD_p = 1.30; SEP = 0.53 %) and linoleic (RPD_p = 1.71; SEP = 0.78 %) acids. However, the results they obtained for the prediction of stearic (RPD_p = 6.26; SEP = 1.19 %) and oleic (RPD_p = 7.91; SEP = 0.90 %) acids were better than those obtained in this study. The oleic acid they found (CV_{val} = 14.31%) exhibited a greater variability than that obtained in our study, which according to Shenk et al. (1997) would enable them to obtain more robust models. In addition, the different sample presentation form (powder), the type of nut and the instrument (monochromator working in the 400-2500 nm range) used by these authors should also be noted.

4. Conclusions

The results obtained demonstrated the viability of NIRS technology for the *in situ* measurement of the fatty acid profile of intact in-shell and shelled almonds, permitting the measurement of the quality and freshness of the nuts at the moment when the product is received by the industry. Nevertheless, further studies are needed in order to improve the robustness of the calibration models.

NIRS technology would enable us to study the evolution of the oleic and linoleic acid contents during the postharvest storage, which is an indicator of the products' freshness, and could act as a support system for decision-making in the management of product batches. The portability of these miniaturized systems means that they can be used for the integrated control of this product, permitting the fatty acid profile to be included on the labelling of the product as an element of nutritional quality, thus satisfying consumer demand for safe, healthy food.

514 The tests carried out in this study have shown that, in order to obtain the best
515 results, the NIRS analysis of the samples must be carried out dynamically, after the
516 almonds have been shelled. Nevertheless, the models developed in this study using in-
517 shell almonds reported promising results for the *in situ* analysis of almond samples before
518 they are shelled and processed.

519

520 **CRediT authorship contribution statement**

521

522 **Miguel Vega-Castellote**: Data acquisition, Methodology, Formal analysis,
523 Investigation, Software, Data curation, Validation, Writing - original draft, Writing -
524 review & editing, Visualization. **Dolores Pérez-Marín**: Conceptualization,
525 Methodology, Validation, Investigation, Resources, Writing – original draft, Writing -
526 review & editing, Visualization, Supervision, Project administration, Funding
527 acquisition. **Irina Torres:** Data acquisition, Formal analysis, Investigation, Software,
528 Data curation, Writing - original draft, Writing - review & editing, Visualization. **María-**
529 **Teresa Sánchez**: Conceptualization, Methodology, Validation, Investigation, Resources,
530 Writing – original draft, Writing - review & editing, Visualization, Supervision, Project
531 administration, Funding acquisition.

532

533 **Declaration of Competing Interest**

534

535 The authors declare that they have no known competing financial interests or
536 personal relationships that could have influenced the work reported in this paper in any
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538

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716

717 **Table 1**

718 Technical features of the diode-array and LVF spectrophotometers.

Property	Instrument	
	Aurora	MicroNIR^{TM} Pro 1700
Detector type	256-pixel InGaAs detector	128-pixel InGaAs photodiode array
Dispersion element	Diode-array	Linear variable filter
Wavelength range (nm)	950-1650	908-1676
Resolution (nm)	2	6.2
Sampling integration time (ms)	6.57	11
Weight (kg)	2	$64 \cdot 10^{-3}$
Analysis mode	Reflectance	Reflectance

719

720 **Table 2**

721 Fisher's test results for the analysis of the STD_{limit} values for the two analysis modes

722 carried out.

Instrument	Almond presentation	Analysis mode	[a] SDT_{limit}	F	$F_{critical}$
Diode-array	In-shell	Static	126,528	5.91*	3.18
		Dynamic	52,026		
	Shelled	Static	49,461	7.49*	3.18
		Dynamic	18,068		
Linear variable filter	In-shell	Static	169,326	10.60*	3.18
		Dynamic	52,014		
	Shelled	Static	96,347	4.67*	3.18
		Dynamic	44,605		

723 [a] standard deviation limit.

724 * Significant differences ($P < 0.05$)

725

726 **Table 3**

727 Number of samples, range, mean, standard deviation (SD) and coefficient of variation

728 (CV) for the fatty acids studied in the calibration and validation sets.

Acid (%)	Set	N	Range	Mean	SD	CV (%)
Palmitic	Calibration	106	5.32-7.70	6.52	0.61	9.36
	Validation	35	5.34-7.40	6.54	0.52	7.95
Stearic	Calibration	106	1.47-3.39	2.12	0.43	20.28
	Validation	35	1.59-3.30	2.15	0.45	20.93
Oleic	Calibration	106	59.32-76.24	69.78	4.42	6.33
	Validation	35	62.69-75.97	70.15	3.41	4.86
Linoleic	Calibration	106	15.34-29.98	20.64	3.73	18.07
	Validation	35	15.60-25.63	20.23	2.84	14.04

729

730

731 **Table 4**

732 Calibration statistics for the best equations obtained for the prediction of saturated

733 (palmitic and stearic) fatty acids in in-shell and shelled almonds.

Acid (%)	Instrument	Almond presentation	Analysis mode	Mean	[a] LV	[b] R^2_{cv}	[c] SECV*	[d] RPD_{cv}
Palmitic	Diode-array	In-shell	Static	6.52	5	0.51	0.43^{a}	1.43
			Dynamic	6.48	6	0.66	0.35^{b}	1.73
		Shelled	Static	6.50	10	0.77	0.29^{c}	2.09
			Dynamic	6.51	11	0.83	0.26^{c}	2.40
	Linear variable filter	In-shell	Static	6.52	7	0.41	0.47^{a}	1.30
			Dynamic	6.52	9	0.49	0.44^{a}	1.40
		Shelled	Static	6.51	8	0.82	0.26^{c}	2.36
			Dynamic	6.52	7	0.82	0.26^{c}	2.33
Stearic	Diode-array	In-shell	Static	2.11	6	0.57	0.28^{a}	1.52
			Dynamic	2.08	7	0.66	0.23^{bc}	1.73
		Shelled	Static	2.11	12	0.58	0.27^{ab}	1.55
			Dynamic	2.11	13	0.79	0.19^{d}	2.16
	Linear variable filter	In-shell	Static	2.08	5	0.57	0.25^{abc}	1.52
			Dynamic	2.10	7	0.60	0.26^{ab}	1.59
		Shelled	Static	2.12	9	0.59	0.27^{ab}	1.56
			Dynamic	2.10	7	0.70	0.22^{cd}	1.83

734 * The coincidence of any of the superscript letters in the different SECV values of the same fatty acid indicates that no
735 significant differences were found ($P > 0.05$) between those values. [a] LV: Number of latent variables; [b] R^2_{cv}: coefficient
736 of determination of cross validation; [c] SECV: standard error of cross validation; [d] RPD_{cv}: residual predictive deviation
737 for cross validation.

738

739 **Table 5**

740 Calibration statistics for the best equations obtained for the prediction of

741 monounsaturated and polyunsaturated fatty acids in in-shell and shelled almonds.

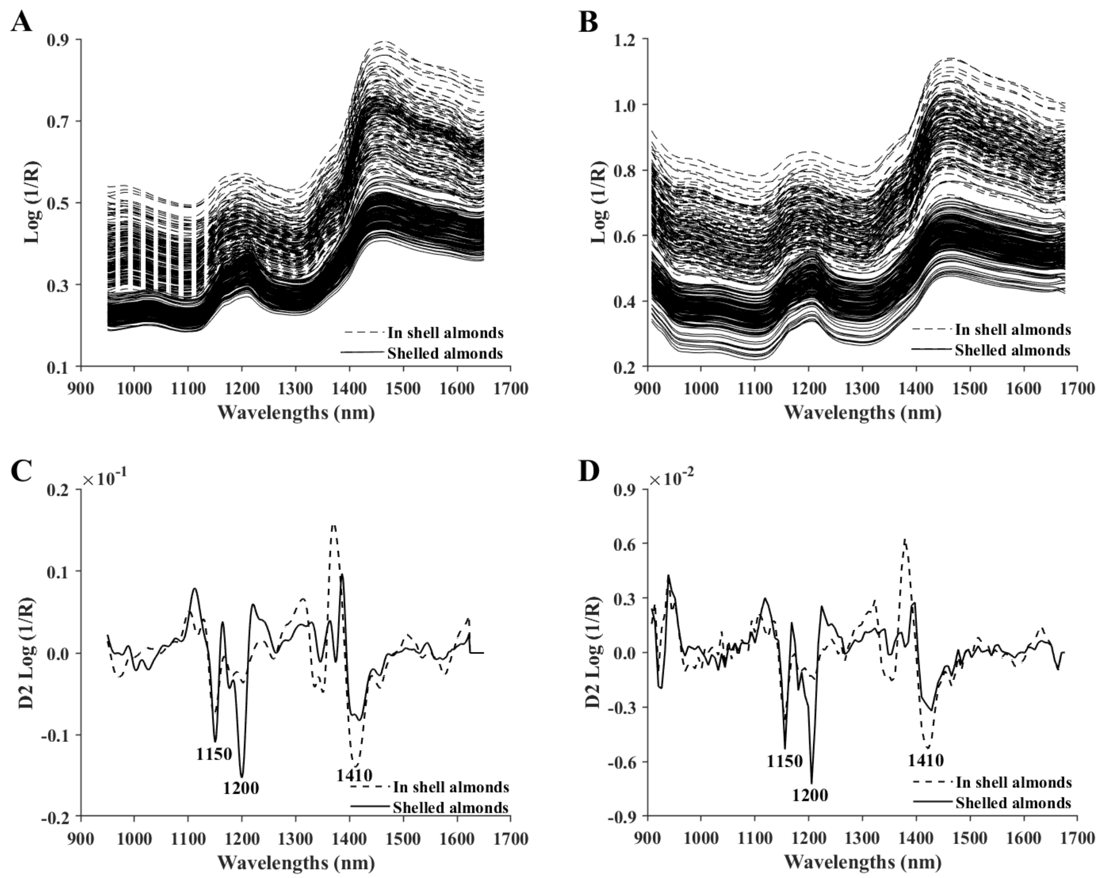
Acid (%)	Instrument	Almond presentation	Analysis mode	Mean	[a] LV	[b] R^2_{cv}	[c] SECV*	[d] RPD_{cv}
Oleic	Diode-array	In-shell	Static	70.11	7	0.68	2.40[a]	1.77
			Dynamic	70.11	6	0.75	2.14[a]	2.02
		Shelled	Static	69.92	8	0.75	2.19[a]	1.99
			Dynamic	69.81	13	0.94	1.11[c]	3.98
	Linear variable filter	In-shell	Static	69.98	8	0.68	2.51[a]	1.78
			Dynamic	69.90	7	0.73	2.32[a]	1.91
		Shelled	Static	70.02	8	0.73	2.20[a]	1.92
			Dynamic	69.86	9	0.87	1.59[b]	2.78
Linoleic	Diode-array	In-shell	Static	20.33	8	0.65	2.09[a]	1.70
			Dynamic	20.35	6	0.76	1.79[ab]	2.03
		Shelled	Static	20.66	16	0.76	1.86[ab]	2.02
			Dynamic	20.63	11	0.93	1.00[d]	3.77
	Linear variable filter	In-shell	Static	20.44	9	0.72	1.97[ab]	1.88
			Dynamic	20.53	9	0.78	1.75[b]	2.11
		Shelled	Static	20.42	8	0.68	1.99[ab]	1.77
			Dynamic	20.58	9	0.86	1.41[c]	2.65

742 * The coincidence of any of the superscript letters in the different SECV values of the same fatty acid indicates that no
743 significant differences were found ($P > 0.05$) between those values. [a] LV: Number of latent variables; [b] R^2_{cv}: coefficient
744 of determination of cross validation; [c] SECV: standard error of cross validation; [d] RPD_{cv}: residual predictive deviation
745 for cross validation.

746

747 **Fig. 1.** Log (1/R) spectra for in-shell and shelled almonds analysed in dynamic mode using the
748 diode-array (A) and the linear variable filter (B) spectrophotometers and D_2 Log(1/R) mean
749 spectra for in-shell and shelled almonds analysed in dynamic mode using the diode-array (C) and
750 the linear variable filter (D) spectrophotometers.

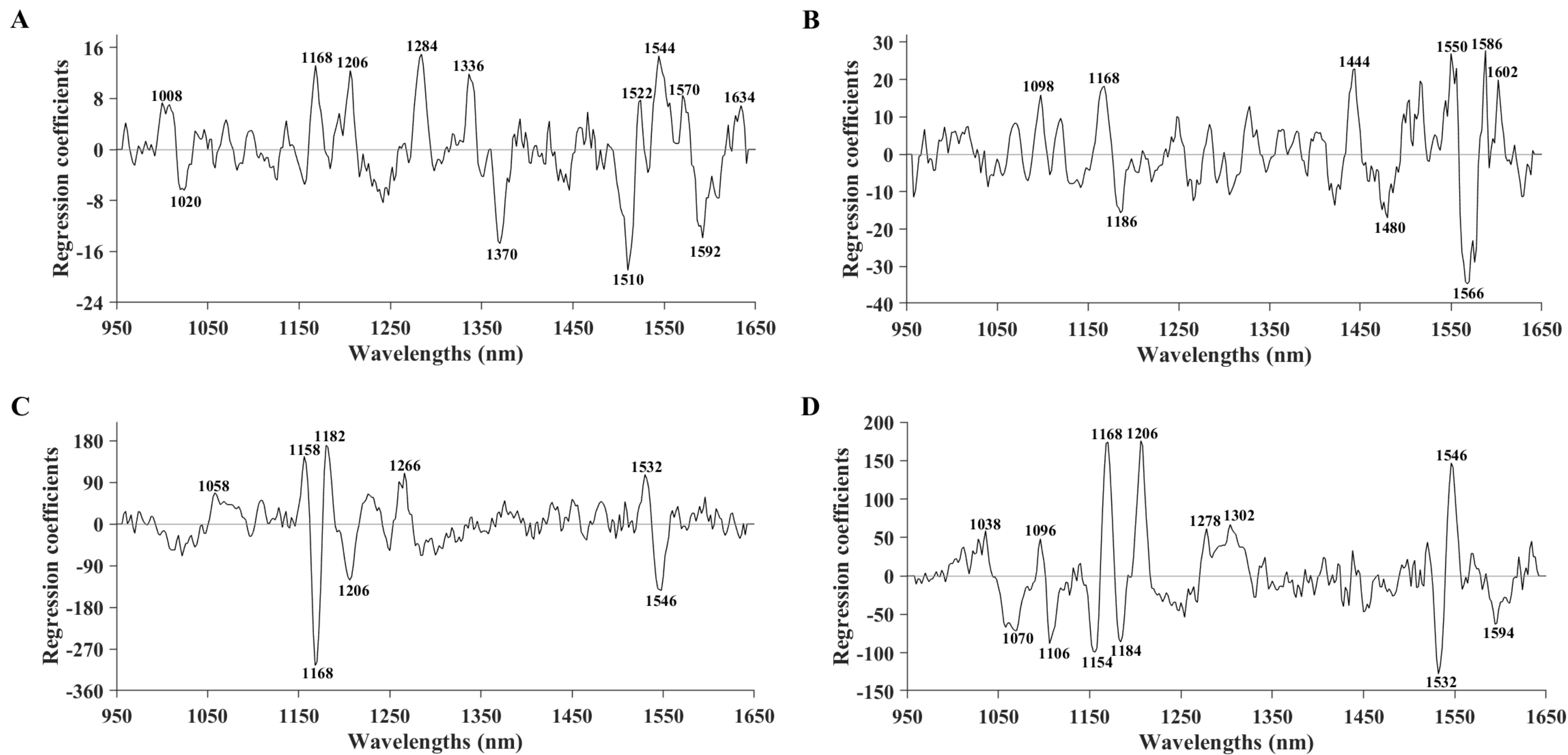
751



752

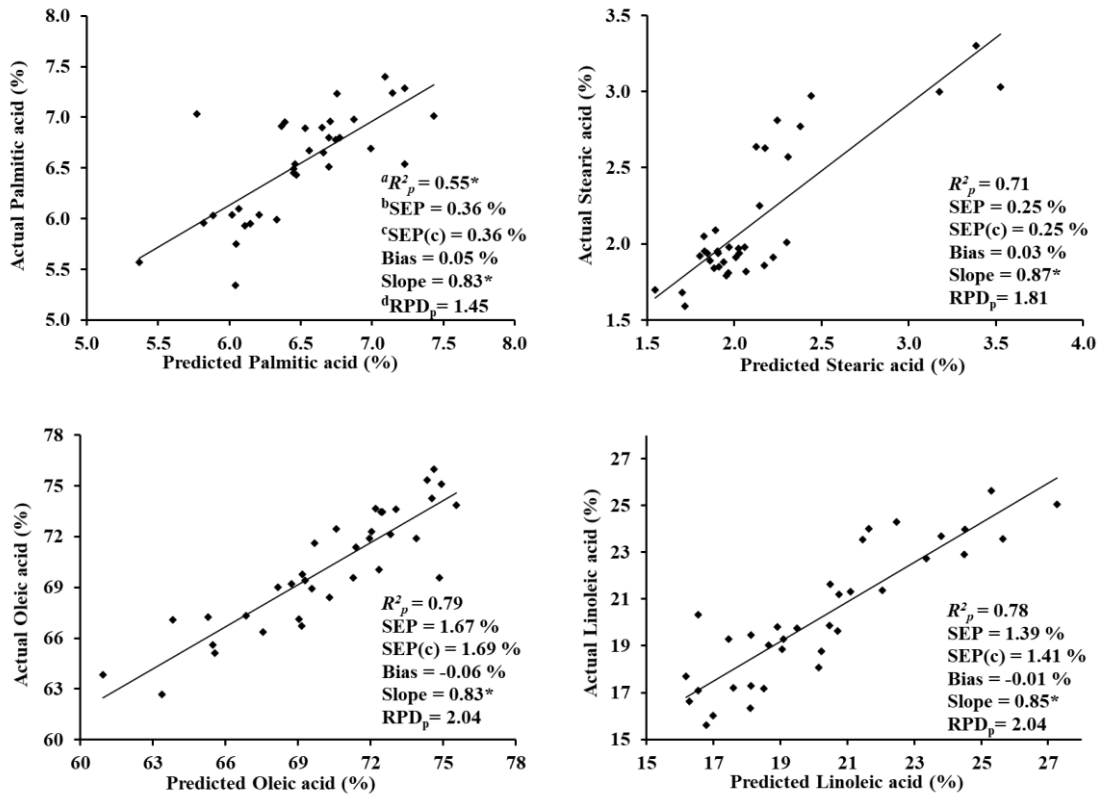
753 **Fig. 2.** Regression coefficients for the best calibration models for the prediction of palmitic (A), stearic (B), oleic (C) and linoleic (D) acids in shelled almonds

754 analysed with the diode-array instrument in dynamic mode.



755

756 **Fig. 3.** Actual *versus* predicted data for the validation of the best models for the prediction of
757 palmitic, stearic, oleic and linoleic acids in in-shell almonds analysed with the diode-array
758 instrument in dynamic mode.



759 [a] R^2_p, coefficient of determination for prediction.

760 [b] SEP, standard error of prediction.

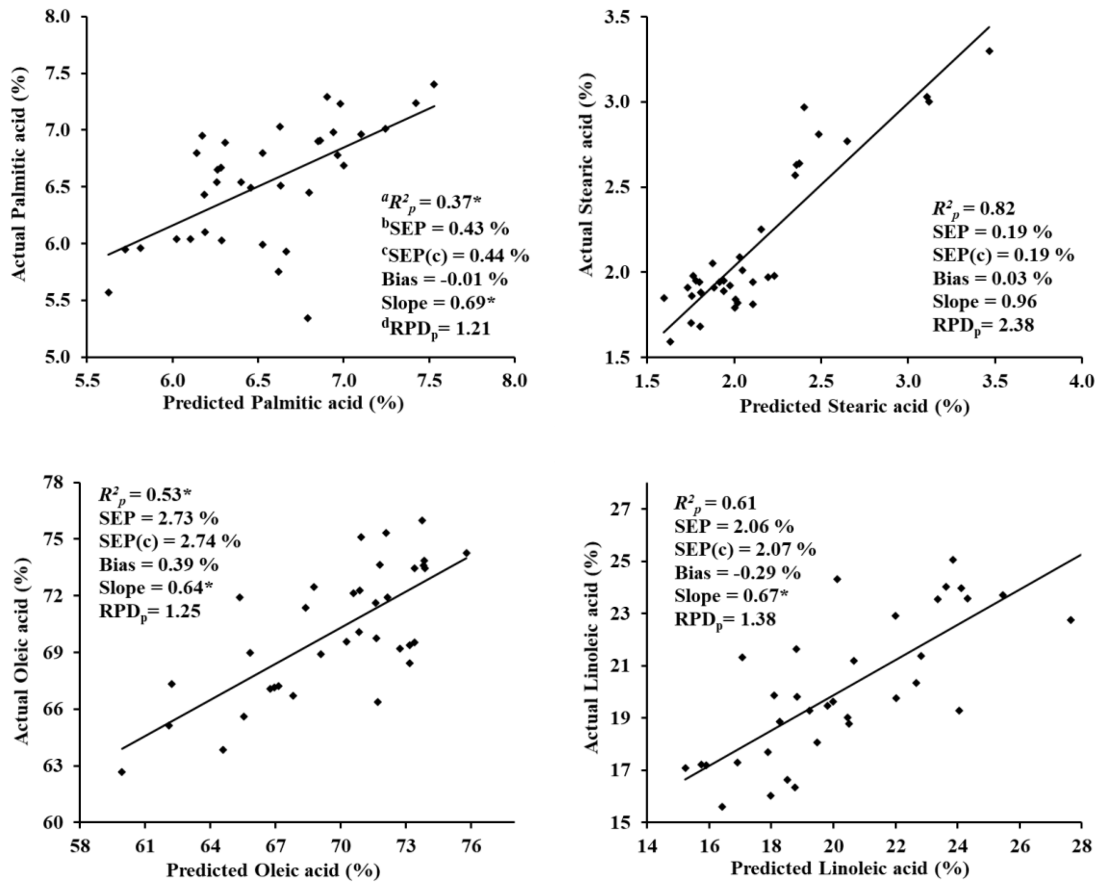
761 [c] $SEP_{(c)}$, standard error of prediction corrected for bias.

762 [d] RPD_p, residual predictive deviation for prediction.

763 * Do not meet the validation requirements (Windham et al., 1989).

764

765 **Fig. 4.** Actual *versus* predicted data for the validation of the best models for the prediction of
766 palmitic, stearic, oleic and linoleic acids in in-shell almonds analysed with the LVF instrument in
767 dynamic mode.



768 [a] R^2_p, coefficient of determination for prediction.

769 [b] SEP, standard error of prediction.

770 [c] $SEP_{(c)}$, standard error of prediction corrected for bias.

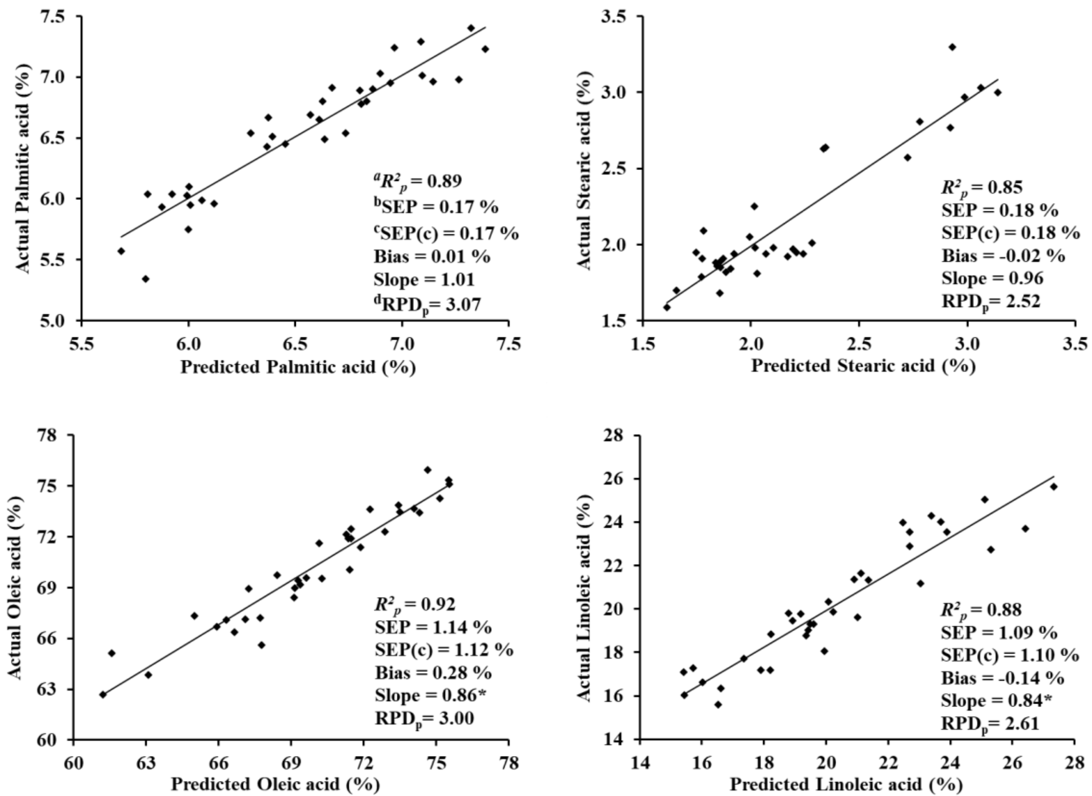
771 [d] RPD_p, residual predictive deviation for prediction.

772 * Do not meet the validation requirements (Windham et al., 1989).

773

774

Fig. 5. Actual *versus* predicted data for the validation of the best models for the prediction of palmitic, stearic, oleic and linoleic acids in shelled almonds analysed with the diode-array instrument in dynamic mode.



[a] R^2_p, coefficient of determination for prediction.

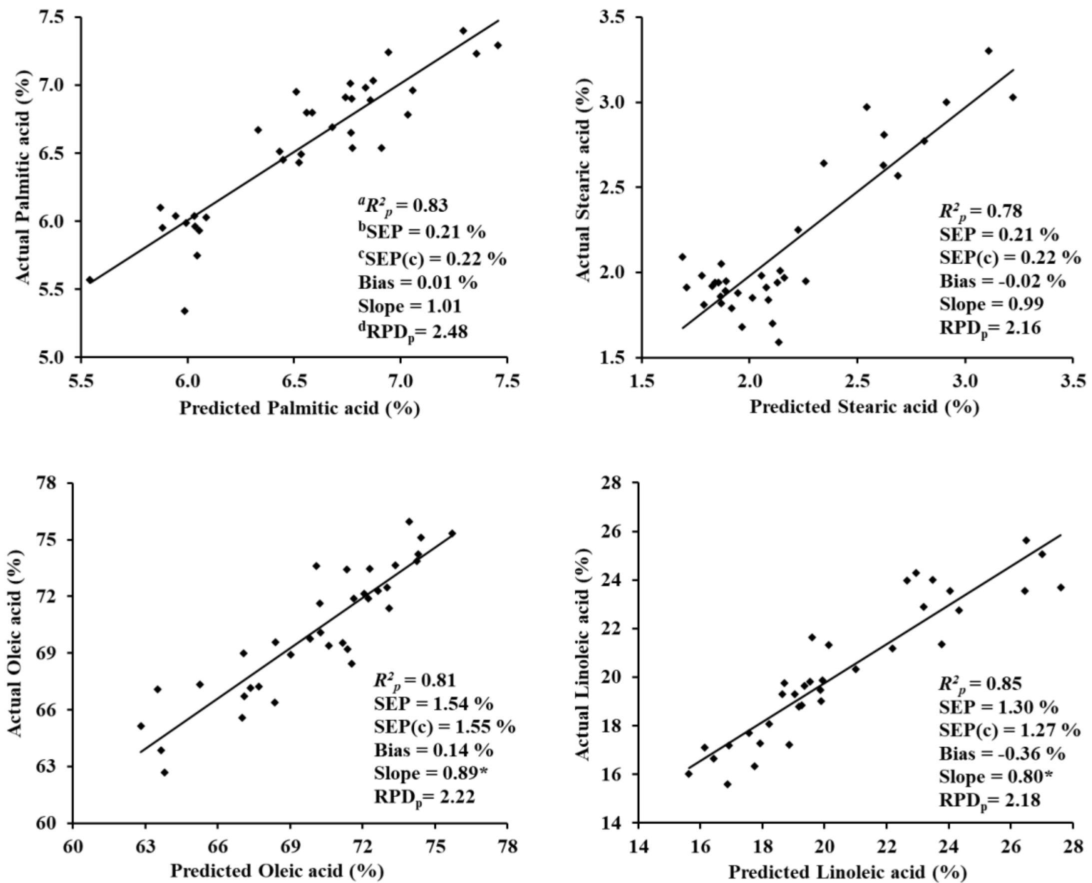
[b] SEP, standard error of prediction.

[c] $SEP_{(c)}$, standard error of prediction corrected for bias.

[d] RPD_p, residual predictive deviation for prediction.

* Do not meet the validation requirements (Windham et al., 1989).

784 **Fig. 6.** Actual *versus* predicted data for the validation of the best models for the prediction of
785 palmitic, stearic, oleic and linoleic acids in shelled almonds analysed with the LVF instrument in
786 dynamic mode.
787



788 [a] R^2_p, coefficient of determination for prediction.

789 [b] SEP, standard error of prediction.

790 [c] $SEP_{(c)}$, standard error of prediction corrected for bias.

791 [d] RPD_p, residual predictive deviation for prediction.

792 * Do not meet the validation requirements (Windham et al., 1989).

793