

1 **Monitoring quality and safety assessment of summer squashes along**
2 **the food supply chain using Near Infrared sensors**

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4 Irina Torres^a, María-Teresa Sánchez^{a,*}, José-Antonio Entrenas^a, Ana Garrido-Varo^b,
5 Dolores Pérez-Marín^{b,*}

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8 ^a *Department of Bromatology and Food Technology, University of Cordoba, Campus of*
9 *Rabanales, 14071 Córdoba, Spain.*

10 ^b *Department of Animal Production, University of Cordoba, Campus of Rabanales,*
11 *14071 Córdoba, Spain.*

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15 *Corresponding authors. Tel.: +34 957 212576; fax: 34 957 212000

16 *E-mail addresses:* teresa.sanchez@uco.es (M.T. Sánchez) or dcperez@uco.es (D.
17 Pérez-Marín).

18

19 **Abstract**

20 Currently, there is growing interest from the producers and the vegetable processing
21 industry to use from farm to fork, non-destructive analysis techniques as a routine step
22 to ensure the quality and safety of horticultural products. This interest, coinciding with
23 the development of new instruments adapted for use both in the field and on the
24 production line, has led to Near Infrared Spectroscopy (NIRS) becoming an
25 increasingly practicable option for meeting the demand. The aim of this work was to
26 develop and optimize NIRS analysis methodology using two spectrophotometers: the
27 first is the MicroNIR™ 1700, a manual, portable instrument based on Linear Variable
28 Filter (LVF) technology, ideally suited to analysing horticultural products in the field,
29 and the other is the Matrix-F, based on Fourier Transform (FT) NIR technology and
30 suitable for online analysis in the processing industries. A total of 230 summer squashes
31 were used to predict the quality (dry matter and soluble solid content) and safety (nitrate
32 content) parameters. For the MicroNIR™ 1700, the comparison between the equations
33 developed confirmed that taking of point spectra (static mode) was the most suitable
34 way of analysis to measure both the quality and safety parameters. In the case of the
35 Matrix-F instrument, it was confirmed that a single spectrum taken online for the intact
36 product as it moves on the conveyor belt (dynamic mode) is enough to establish the
37 product's quality and safety during industrial processing, thus allowing it to be
38 incorporated easily and conveniently into the production line.

39

40 *Keywords:* Summer squash; NIRS technology; *In situ* NIR analysis optimization; Safety
41 and quality parameters; monitoring the food chain with sensors

42

43 **1. Introduction**

44

45 Summer squash, due to its high content of vitamins, fibre, minerals and trace
46 elements and its extremely low caloric value, is an ingredient widely used in the baby
47 food processing industry (Paris, 2008). Despite the numerous benefits accruing from the
48 consumption of this vegetable, recently there has been growing awareness among
49 consumers about the presence of nitrates in summer squash and about the risk involved
50 in an excessive intake of these substances, which are reduced to nitrites in the human
51 body (Mensinga et al., 2003).

52 Despite the introduction of established codes of good agricultural practices, in
53 certain cases, some producers fail to reduce the levels of nitrates present in vegetables,
54 mainly due to the influence of climate, and particularly, to the amount of light.
55 Although the high light intensity typical of summer crops favours the plant's
56 metabolism by fixing nitrogen in the form of organic nitrogenous compounds, such as
57 aminoacids, proteins, chlorophyll, etc., and reducing its nitrate content, low light
58 intensity (e.g. in winter crops) favours the presence of higher concentrations of nitrates
59 (Blom-Zandstra, 1989).

60 The European Union is aware of these problems and has established maximum
61 limits for nitrates content in summer squash according to their final uses (OJEU, 2011).
62 As a result, both producers and the infant food industry must understand that only those
63 summer squashes with a nitrate content below 200 mg kg^{-1} can be considered suitable
64 for the production of baby food products. In addition, these regulations stress the need
65 for producers and the agri-food industry to employ non-destructive analysis
66 technologies in the handling and processing industries, which may be used both *in-situ*

67 (in the field) and online, and which allow to measure the level of nitrates present in
68 summer squash and, therefore, establish its final use, in a matter of seconds.

69 NIR spectroscopy is a fast, accurate, non-destructive and reliable technology
70 which is ideal for this purpose. However, the only scientific article published to date on
71 the non-destructive measurement of quality parameters (dry matter and soluble solid
72 content) and safety (content of nitrates) in summer squash using NIRS technology has
73 been that of Sánchez et al. (2017), who used a handheld MEMS (micro-electro-
74 mechanical system)-based NIRS digital transform spectrophotometer and performed the
75 NIRS analysis for these parameters *in-situ* on summer squash, exclusively taking of
76 point spectral readings at the equatorial region of the fruits.

77 Recently, a new generation of portable, compact and extremely light-weight
78 NIRS instruments has been developed, ideally suited for use in the field and for taking
79 *in-situ* measurements (Yan and Siesler, 2018). These instruments enable not only to
80 take spectra at any time, but also to analyse the whole surface of the product, thus
81 obtaining more information about the quality and safety of the product to be harvested.

82 Lately, there has also been a growing interest from the vegetable processing
83 industry to incorporate NIR spectroscopy as a routine technique to ensure the quality
84 and safety of the vegetables they process, and this requires the NIRS applications to be
85 developed in advance, simulating the industrial processes of the horticultural industries.
86 One fundamental issue in developing online NIRS applications is to make the correct
87 choice of instrument to be used, which must be robust and stable when subjected to
88 vibrations and thermal variations (Porep et al., 2015; Garrido et al., 2018).

89 In the same way, it should be noted that, regardless of the type of application and
90 instrument used, the routine incorporation of NIRS technology both in the field and in
91 the industry requires a system to be established which includes all the issues related to

92 taking spectra, as well as the selection of the optimal spectral region for each instrument
93 used. For this reason, in the present research, the NIRS analysis methodology has been
94 fine-tuned and optimized to measure the quality and safety parameters in summer
95 squash both *in-situ*/on the plant and online, in the industry. Two new generation of NIR
96 spectrophotometers, one manual, portable instrument based on LVF technology
97 (MicroNIR™ 1700), suitable for use in the field, to analyse the product while it is
98 developing in the plant, and another based on FT-NIR technology (Matrix-F) which can
99 be incorporated into the processing industry of these vegetables, such as in the product
100 sorting areas, and to control the quality and safety standards established by the industry.

101

102 **2. Materials and methods**

103

104 *2.1. Sampling*

105

106 A total of 230 summer squashes (135 less than 400 g in weight and 95 more than
107 400 g, being the minimum, maximum and mean weight of the fruits tested: 78.43 g,
108 1746.49 g and 454.13 g, respectively) (*Cucurbita pepo* subsp. *pepo* cv. Mirza) grown in
109 an open-air field in the district of La Montiola, Santaella (Córdoba, Spain), were weekly
110 harvested at commercial maturity (OJEU, 2008), from mid-May to mid-July 2017,
111 being the number of harvests of 10. Samples were stored under refrigerated conditions
112 (5°C and 85% RH) until the following day, when the analysis was performed. Prior to
113 each measurement, the samples were left to reach room temperature.

114

115 *2.2. Reference data*

116

117 Dry matter, soluble solid content (SSC) and nitrate content were measured
118 following the procedures used by Sánchez et al. (2017). To analyse these parameters in
119 summer squashes weighing more than 400 g, the fruit was cut into three sections: the
120 stem region (upper third of the squash starting at the peduncle), the equatorial region
121 (middle third in the equator of the fruit) and the styler region (lower third of the fruit,
122 starting at the pistil scar). All the analytical measurements were performed in duplicate
123 immediately after NIR spectrum collection and the standard error of the laboratory
124 (SEL) was estimated from these duplicates (Table 1).

125

126 2.3. Obtaining the NIR spectra

127

128 The NIR spectra of summer squashes were collected using two instruments
129 adapted to *in-situ* and online applications, respectively.

130 A handheld LVF instrument (MicroNIR™ 1700 spectrophotometer, JDSU
131 Uniphase Corporation, Milpitas, CA, USA), designed for *in-situ* analysis, in the field or
132 in the industry, was used in reflectance mode ($\log 1/R$). The MicroNIR™ 1700 is a
133 miniaturised near-infrared analyser that relies on a linear variable filter as the dispersion
134 element. This portable miniature spectrometer is extremely light (64 g). The
135 spectrophotometer scans at a constant interval of 6.2 nm, across the NIR wavelength
136 range of 908 to 1676 nm, and its optical window is around 227 mm². The instrument's
137 performance was checked every 10 min. A white reference measurement was obtained
138 using a NIR reflectance standard (Spectralon™) with a 99% diffuse reflectance, while a
139 dark reference was obtained from a fixed point in the room.

140 The online instrument used for the spectrum acquisition was the FT-NIR
141 spectrophotometer Matrix-F (Bruker Optik GmbH, Ettlingen, Germany). This

142 equipment consists of a sensor head with two NIR light sources which illuminate a
143 sampling spot of 10 mm in diameter, attached to the instrument via a 5 m-long fibre
144 optic probe. The spectra were collected in reflectance mode in the spectral range from
145 4000 to 12000 cm^{-1} (834–2502.40 nm), with a resolution of 16 cm^{-1} . Furthermore, the
146 system was equipped with a conveyor belt to move the sample, whose speed was set at
147 10 kHz. An internal white reference was also collected every ten minutes.

148 The spectra taken with the portable LVF instrument were evaluated in two
149 different modes: static – taking of point spectra readings in the centre of the surface of
150 the summer squashes analysed, without the instrument moving during the measurement
151 –, and dynamic, with the sensor being moved during the spectral measurements all
152 along the length of the summer squash being analysed. For both analysis modes, an
153 integration time of 11000 μs and 200 scans per spectrum were set.

154 To take the spectra using the MicroNIRTM 1700 instrument, the summer squash
155 were cut into the three regions mentioned above (stem, equatorial and styler regions),
156 regardless of the weight of the fruit analysed. In the static analysis, 4 spectra were taken
157 at the centre of each of the selected regions, at 90° intervals, so that 12 spectra were
158 obtained per summer squash, while in the dynamic mode analysis, 4 spectra were also
159 taken covering each of the regions analysed and rotating the fruit 90° between
160 measurements, again obtaining 12 spectra per fruit analysed. In the case of summer
161 squashes weighing less than 400 g, 12 spectra were taken per fruit. These 12 spectra
162 were averaged to obtain the mean spectra for each fruit, obtaining a number of spectra
163 similar equal to the number of analysed fruits, in this case 135 spectra.

164 For summer squashes weighing more than 400 g, two strategies were followed:

165 (I) An average was taken of the four spectra corresponding to each of the regions
166 (stem, equatorial and stylar regions) analysed, thus obtaining one single spectrum per
167 region.

168 (II) The 12 spectra taken for each summer squash were averaged, producing one
169 spectrum per fruit.

170 Therefore, for Strategy I, an initial sample group consisting of 420 spectra was
171 obtained, i.e. 95 fruits x 3 regions/fruit x 1 spectrum/region (summer squashes more
172 than 400 g in weight) + 135 fruits x 1 spectrum/fruit (summer squashes less than 400 g
173 in weight), while for strategy II, an initial group of spectra was obtained equal to that of
174 the number of fruits analysed (230 spectra = 95 spectra of summer squashes more than
175 400 g in weight + 135 spectra of summer squashes less than 400 g in weight).

176 With the Matrix-F instrument, the analysis was carried out online as the product
177 was moving on the conveyor belt. The number of scans selected per fruit was 12,
178 covering the entire length of the fruit as it moved on the belt, and, with this instrument,
179 no analysis of different fruit regions was carried out. A total of 4 spectra per fruit were
180 taken, rotating the fruit 90° after each measurement, and different strategies were used
181 to perform their chemometric analysis:

- 182 1. Selecting a single spectrum per summer squash analysed.
- 183 2. Using the average spectrum obtained after taking 2 spectra per summer
184 squash.
- 185 3. Using the average spectrum of the 4 spectra taken for each summer squash.

186 In the case of strategies 1 and 2, the spectra were randomly selected by Matlab v.
187 2015a (The Mathworks, Inc., Natick, Massachusetts, USA).

188 However, regardless of the strategy followed, the total number of spectra used
189 for the development of the predictive models was 230, which was equal to the number
190 of fruits used in this study.

191

192 *2.4. Definition of calibration and validation sets*

193

194 Prior to the development of NIR calibrations, data pre-processing and
195 chemometric treatments were performed using the WinISI software package ver. 1.50
196 (Infrasoft International LLC, Port Matilda, PA, USA).

197 Firstly, the most suitable spectral range for the instruments tested to carry out the
198 quality and safety control of summer squashes was selected. To achieve this, the 1,1,1,1
199 derivation treatment was applied (the first digit being the number of the derivative, the
200 second the gap over which the derivative is calculated, the third the number of data
201 points in a running average or smoothing, and the fourth the second smoothing) without
202 scatter correction, which allows to highlight the areas of the spectrum where the
203 signal/noise ratio is degraded (Hruschka, 2001).

204 Additionally, and in the case of the instrument used to simulate the analysis of
205 summer squash in vegetable sorting lines, the Matrix-F spectrophotometer, a distance of
206 12 cm between the instrument head and the conveyor belt (head-fruit distance was 8 cm
207 and 10 cm for summer squashes weighing both more and less than 400 g, respectively)
208 was established, which remained constant throughout the process of taking spectra,
209 regardless of the type of summer squash analysed. This attempted to simulate the
210 existing conditions in the food processing industry, which uses fruits with different
211 equatorial diameter values in their classification processes, and where the automatic
212 readjustment of the distance between the instrument and the belt, depending on the

213 different equatorial diameters presented by the summer squash analysed, is not possible.
214 Next, principal component analysis was used to study the influence of the interaction of
215 the light with the product when this distance was kept constant, regardless of the
216 equatorial diameter of the fruit analysed.

217 Secondly, the CENTER algorithm was applied to ensure a structured population
218 based on spectral information for the selection of calibration and validation sets. This
219 algorithm performs principal component analysis (PCA) and determines the centre of
220 the population and the distance between each sample and the centre [Mahalanobis
221 distance (GH)]. Samples with a statistical value greater than 4 were considered outliers
222 or anomalous spectra (Shenk and Westerhaus, 1995a). As spectral pre-treatments,
223 Standard Normal Variate (SNV) and Detrending (DT) were used to remove scatter
224 interferences (Barnes et al., 1989), together with the first derivative treatment '1,5,5,1'
225 (Shenk and Westerhaus, 1995b).

226 Once spectral outliers had been removed for each instrument, three of every four
227 samples were selected to form part of the calibration set and the remainder constituted
228 the validation set (Table 1). Thus, for the MicroNIR™ 1700, a calibration group, C1
229 (305 samples) and a validation group, V1 (107 samples), for strategy I were established.
230 For the Matrix-F, the calibration group (C2) and validation group (V2) were made up of
231 169 samples and 53 samples, respectively, for each of the fruit spectra-taking strategies
232 tested (strategies 1, 2 and 3). Finally, to analyse the results obtained with the two
233 instruments tested, the same calibration groups (C2 set) and validation (V2 set) were
234 selected for strategy II for the MicroNIR™ 1700 as for the Matrix-F.

235

236 *2.5. Construction and validation of prediction models using a linear regression strategy*

237

238 Modified partial least squares (MPLS) regression (Shenk and Westerhaus,
239 1995a) was used to obtain NIR calibration models for the prediction of quality and
240 safety parameters in summer squashes using the MicroNIR™ 1700 (calibration sets C1
241 and C2) and the Matrix-F (calibration set C2). All regression equations were obtained
242 using SNV + DT for scatter correction (Barnes et al., 1989) and different derivative
243 mathematical treatments were tested: 1,5,5,1; 2,5,5,1; 1,10,5,1 and 2,10,5,1 (Shenk and
244 Westerhaus, 1995b). Calibration models were constructed eliminating physical-
245 chemical outlier samples, if necessary.

246 The statistical parameters employed to select the best equations using MPLS
247 were the coefficient of determination for calibration (R^2_c), the standard error of
248 calibration (SEC), the coefficient of determination for cross validation (R^2_{cv}) and the
249 standard error of cross validation (SECV). Furthermore, the Residual Predictive
250 Deviation (RPD_{cv}) for cross-validation was calculated as the ratio of the standard
251 deviation (SD) of the reference data to the SECV. This statistical parameter enables
252 SECV to be standardized, facilitating the comparison of results obtained with sets of
253 different means (Williams, 2001).

254 For strategy I, once the best predictive model for each parameter analysed (dry
255 matter, SSC and nitrate content) using the instrument MicroNIR™ 1700 in static and
256 dynamic modes were selected by statistical criteria - and prior to external validation -,
257 tests were run for significant differences between the predictive capacity of the quality
258 and safety models developed for each parameter. This approach was used to identify the
259 most suitable analysis mode for measuring *in-situ* quality and safety in summer squash.
260 The SECV values for the best equations obtained for both analysis modes were
261 compared using Fisher's F test (Massart et al., 1988; Naes et al., 2002). Values for F
262 were calculated as:

263
$$F = \frac{(SECV_2)^2}{(SECV_1)^2}$$

264 where $SECV_1$ and $SECV_2$ are the standard error of cross validation of two
265 different models and $SECV_1 < SECV_2$. F is compared to $F_{critical (1-P, n-1, n-1)}$ read from the
266 table with $P = 0.05$ and $n-1$ degrees of freedom. If F is higher than $F_{critical}$, the two
267 $SECV$ values are significantly different.

268 For the online instrument (Matrix-F), different predictive models, without the
269 elimination of physical-chemical outliers ~~during their development~~, were developed to
270 test the optimum number of spectra (1, 2 or 4 spectra) per fruit that must be taken ~~in~~
271 ~~order~~ to obtain robust models so as to establish an NIR analysis methodology in
272 summer squash which ultimately could be transferred to the horticultural processing
273 industry. The $SECV$ values for the best equations obtained without the elimination of
274 physical-chemical outliers during the development of the models for each parameter
275 with different numbers of spectra per fruit were also compared using Fisher's F test
276 (Massart et al., 1988; Naes et al., 2002). Because in this study several $SECV$ values
277 were compared, a $SECV_{confidence\ limit}$ was calculated using the following formula:
278 $SECV_{confidence\ limit} = SECV_{min} \cdot \sqrt{F_{critical}}$ where $SECV_{min}$ is the smallest $SECV$.
279 Consequently, none of the models which had a $SECV$ between $SECV_{min}$ and
280 $SECV_{confidence\ limit}$ were significantly different ($P < 0.05$).

281 Once the best analysis strategy (optimal number of spectra per fruit) was chosen
282 to measure online the quality and safety parameters of summer squash using the Matrix-
283 F equipment, optimization of the NIR models was carried out for the parameters
284 studied.

285 Finally, once the best equations for each of the instruments used were selected
286 according to the statistical criteria and once both the best strategy of taking spectra was
287 chosen (static or dynamic mode) when using the MicroNIR™ 1700, and the optimal

288 number of spectra per fruit was established when using the Matrix-F, these models were
289 subjected to an external validation process using the corresponding validation group for
290 each instrument (V1 for the MicroNIR™ 1700 and V2 for the Matrix-F), following the
291 protocols outlined by Windham et al. (1989).

292 Lastly, after analysing the results obtained and in order to carry out the analysis
293 of the influence of the number of samples contained in the calibration group on the
294 robustness of the developed models, new predictive models were developed for the
295 three parameters analysed. That is, the same calibration group C2 (N = 169 samples)
296 (Table 1) was used for the two instruments tested. Next, the two instruments were
297 compared using the RPD_{cv} values obtained for the three parameters analysed using
298 Fisher's F test, as mentioned above. It should be noted that, since the
299 spectrophotometers used were originally designed for different applications, *in-situ* and
300 online analysis, the aim of this comparison was not the choice of instrument, but the
301 availability of the results which can be used as a reference to estimate the robustness of
302 the models obtained.

303

304 **3. Results and discussion**

305

306 *3.1. Optimization of the spectral analysis*

307

308 Prior to performing the Principal Component Analysis (PCA) and developing
309 the prediction models, the region of the spectra affected by noise was determined for
310 both instruments. This aspect is especially relevant for the Matrix-F, since with this
311 equipment, the spectral signal is transmitted by fibre optics, which commonly produce
312 noise on extreme wavelengths (Garrido-Varo et al., 2018).

313 Figures 1a and 1b show the first derivative spectra of summer squashes for the
314 MicroNIR™ 1700 and Matrix-F instruments, respectively. In the case of the
315 MicroNIR™ 1700, no areas of the spectrum needed to be removed due to the presence
316 of noise, and the final range was between 908–1676 nm (Fig. 1a). On the other hand, in
317 the case of the Matrix-F, the regions between 834–1075 nm and between 2360–2502
318 nm were removed due to the presence of noise in the spectrum (Fig. 1b).

319 The representation of the scores of PC1 *versus* PC2 (Fig. 2) of the spectra taken
320 with the Matrix-F instrument following strategy 3 (with a mean spectrum of 4 spectra
321 per fruit), allowed to draw a clear distinction between the two groups of summer
322 squashes analysed (more and less than 400 g). Because it is not possible to adjust
323 automatically the height of the equipment based on the equatorial diameter of the fruit
324 analysed while the industrial process is in motion, this study demonstrates the
325 importance of optimizing the analysis distance between the head and conveyor belt, so
326 that the difference in the amount of light which the fruits of different values of
327 equatorial diameter are exposed to is minimal. Besides, the application of spectral pre-
328 treatments could reduce these differences (Workman, 2008).

329 In the case of solid products which are analysed intact, as is the case of summer
330 squash, part of the incident radiation can be lost due to the different ways in which the
331 light interacts with the sample. These include phenomena such as specular reflectance
332 or dispersed radiation, which can lead to the responses not containing information or not
333 reaching the detectors. These phenomena can be influenced by different factors such as
334 the colour, brightness and shape of the sample, among others (Dahm and Dahm, 2001;
335 Jie et al., 2014).

336

337 *3.2. Characterisation of calibration and validation sets*

338

339 To sort the samples according to the Mahalanobis distance (GH), the CENTER
340 algorithm was applied to the spectral databases. Samples with GH values greater than 4
341 were considered outliers.

342 In the case of the MicroNIR™ 1700 for Strategy I (initial sample set = 420
343 spectra), a total of 8 outliers (4 corresponding to summer squash weighing more than
344 400 g y 4 corresponding to fruit weighing less than 400 g) were removed.

345 For the Matrix-F instrument, regardless of the number of spectra taken per
346 sample, the number of outliers was 8 (2 corresponding to summer squash weighing
347 more than 400 g and 6 to fruit weighing less than 400 g), and these were also removed.
348 For the MicroNIR™ 1700 by using stratagem II, in order to obtain the same calibration
349 and validation sets as when using the Matrix-F, the same samples considered as outliers
350 for the Matrix-F were removed for the portable equipment.

351 After removing the outliers, the set for the MicroNIR™ 1700 (strategy I) was
352 split into calibration (C1 = 305 samples) and validation (V1 = 107 samples), while for
353 the Matrix-F and the MicroNIR™ 1700 (strategy II) the sample set was divided into
354 calibration (C2 = 169 samples) and validation (V2 = 53 samples) sets, respectively. The
355 statistical data for the parameters analysed (i.e. number of samples, range, mean, SD
356 and CV) for each instrument are shown in Table 1.

357 For each of the parameters measured in this study, the ranges of the calibration
358 groups included those for validation.

359 For both instruments, the parameter with the greatest variability was the nitrate
360 content, with a CV between 82.71% and 83.42% and between 76.58% and 91.95% for
361 the calibration and validation groups, respectively. This variability was due to the wide
362 range and standard deviation obtained for this parameter because the samples were

363 taken during the summer squash harvest period, with the nitrate content of the fruits
364 decreasing as the harvest period progressed, due to the cessation of fertilization.
365 However, for dry matter ($CV_c = 15.78-16.43\%$, $CV_p = 14.50-14.82\%$) and SSC ($CV_c =$
366 $11.35-11.42\%$, $CV_p = 11.01-12.02\%$) the groups show less variability, because the fruits
367 were collected at the point of commercial maturity.

368

369 *3.3. Development of predictive models and choice of the best analysis mode to predict* 370 *quality and safety parameters using the MicroNIR™ 1700 instrument*

371

372 The calibration statistical parameters for the best models obtained for each
373 parameter analysed in static and dynamic modes using the MicroNIR™ 1700
374 instrument and the C1 set, including the F values obtained from the comparison
375 between the SECV values for each mode of analysis are shown in Table 2.

376 Non-significant differences ($P > 0.05$) were obtained between the dynamic and
377 static modes to predict all the quality (dry matter and SSC) and safety (nitrate content)
378 parameters. The lowest SECV values were obtained in the static mode analysis for all
379 the parameters except for SSC, where a slightly lower SECV value was obtained in the
380 dynamic mode (Table 2).

381 Although it could be argued that the dynamic mode analysis resulted in a better
382 fit, as it covered the whole area of the fruits analysed and collected more information
383 about it, because the fact that the surface of the squash is not flat, it can lead to greater
384 difficulty and associated error when taking the spectra. For this reason, the results
385 showed non-significant differences between the static analysis of the fruits and the
386 dynamic mode. It is also important to note that, given the shape of the summer squash
387 fruits, taking of point spectra readings of the fruit before harvest time, while it is

388 developing on the plant, would be an easier way to use the instrument in the field. In
389 addition, it would make it easier for the farmers to take spectra simpler, quicker and
390 more comfortable.

391 After choosing the static analysis mode as the most suitable for the *in-situ*
392 analysis of summer squash using the MicroNIR™ 1700 instrument, the statistics of the
393 calibration models obtained with this analysis mode were analysed in more detail (Table
394 2).

395 According to Shenk and Westerhaus (1996), the model developed for the dry
396 matter parameter presented a predictive capacity which enabled to discriminate between
397 high, medium and low values of the parameter, while for SSC it was only possible to
398 differentiate between high and low values.

399 Only one work has been found in the literature in which NIRS technology was
400 used to predict parameters such as dry matter and SSC. In Sánchez et al. (2017), a
401 portable equipment based on MEMS technology in the spectral range 1600-2400 nm
402 was used to measure dry matter and SSC, and the predictive capacity of models (RPD_{cv}
403 = 1.71 and 1.76, respectively) was very similar to the one obtained in the present study
404 (Table 2).

405 As regards to the nitrate content parameter, the predictive capacity of the model
406 obtained allowed to distinguish between high, medium and low values for this
407 parameter (Shenk and Westerhaus, 1996). The results obtained by Sánchez et al. (2017)
408 for this parameter using the portable instrument Phazir 2400 and with a calibration
409 group within a range of 30.00–1074.00 mg L⁻¹ (SD = 288.68), were superior (RPD_{cv} =
410 2.42) to those obtained in this work. However, it should be noted that the Phazir 2400
411 has a different spectral range compared to the instrument used in the present work
412 (1600-2400 nm) and this instrument is no longer being used. The recent development of

413 applications using the new manual, portable, light-weight equipment with a wider
414 optical analysis window, such as the MicroNIR™ 1700, has therefore been of great use
415 to producers to determine the final destination of the harvested product.

416

417 *3.4. Selection of the best spectrum capture strategy for online NIR analysis with the* 418 *Matrix-F instrument*

419

420 Table 3 shows the SECV values of the best calibration models obtained using
421 the Matrix-F instrument (C2 set), as well as different strategies for the number of
422 spectra to be taken (1, 2 and 4 spectra per fruit analysed), for each of the parameters
423 studied. In order to compare the three strategies used to take spectra, the calibration
424 models for the different parameters in the study were carried out without eliminating the
425 physical/chemical outliers during their development. That is, the values for mean, range
426 and standard deviation for each parameter tested were the same, regardless of the
427 number of spectra taken per fruit.

428 No significant differences were found, for any of the parameters analysed,
429 between the SECV values of the predictive models developed for the different strategies
430 tested. Therefore, taking into account the results obtained, and in order to facilitate the
431 use of the NIR spectroscopy in the industries where summer squashes are handled, the
432 simplest, quickest way to measure the quality and safety parameters online, during the
433 process of selection and classification of fruits would be to take only one spectrum per
434 fruit, which makes this strategy highly suitable for use in sorting lines.

435 The results obtained agree with those reported by McCarthy and Kemeny
436 (2008), who showed that when using FT-NIR instruments, due to the improved

437 signal/noise ratio in these instruments, a smaller number of spectra per analysed sample
438 was needed for the measurement to yield relevant information.

439

440 *3.5. Development of new models for online prediction of quality and safety parameters*
441 *in summer squash using the Matrix-F*

442

443 After selecting the optimum number of spectra to be taken per fruit using the
444 Matrix-F instrument (1 spectrum/fruit), new predictive models were developed for the
445 parameters analysed, and these were optimized by removing any physicochemical
446 outliers. Table 4 shows the calibration statistical data for the best models developed to
447 predict dry matter, SSC and nitrate content in intact summer squash analysed online
448 with this instrument.

449 For dry matter, the model developed enabled to differentiate between high,
450 medium and low values, while for SSC, the model only distinguished between high and
451 low values (Shenk and Westerhaus, 1996). These results are highly relevant because the
452 spectra were taken on the moving fruit and that the models were developed using a
453 single spectrum per sample, which shows the usefulness of the Matrix-F for measuring
454 quality parameters online.

455 No data has been published on the best approach to use when online
456 measurements of dry matter and SSC are taken in intact summer squashes. However, Jie
457 et al., (2014) predicted SSC in watermelon with NIR equipment (Ocean Optics Inc.,
458 USA) adapted for online use. In transmittance mode in the spectral range 200–1100 nm:
459 RPD_p value of 1.32 was obtained, thus illustrating the greater difficulty of taking quality
460 parameter measurements while the fruit is moving.

461 The results obtained for nitrate content were limited for routine use ($RPD_{cv} =$
462 1.36). The reason for this may be that, for parameters such as nitrates, which are found
463 in very low concentrations in summer squash (i.e., in parts per million), the fact that no
464 contact is made with the fruit when taking the measurement may affect the results for
465 low concentration parameters. Thus, it is essential that a large group of samples must be
466 used in order to produce a robust calibration (Sánchez et al., 2011), and for future work,
467 a much larger number of fruits must be provided.

468 It is also important to note that in this research work, spectra were also taken in
469 summer squash (1 spectrum per fruit) with the Matrix-F instrument while the conveyor
470 belt had stopped (data not shown). For this mode of analysis, the predictive capacity of
471 the models obtained for each parameter ($RPD_{cv} = 2.00$, $RPD_{cv} = 1.63$ and $RPD_{cv} = 1.27$,
472 for dry matter, SSC and nitrate content, respectively) was similar to that obtained in the
473 dynamic mode (Table 4). These results are of particular interest for the industry, since
474 they reinforce the potential of the Matrix-F equipment to be used as a tool to measure
475 quality parameters in moving production lines.

476

477 *3.6. External validation*

478

479 Validations of the best calibration models obtained for the MicroNIR™ 1700
480 (calibration set C1) and Matrix-F (calibration set C2) were performed using the sets V1
481 and V2, respectively, for the two instruments tested.

482 For the MicroNIR™ 1700 instrument and the V1 set, according to the validation
483 protocol established by Windham et al. (1989), the models constructed for predicting all
484 the parameters analysed met the validation requirements in terms of $SEP_{(c)}$, bias and
485 slope (Fig. 3a). Although the R^2_p values did not attain the recommended minimum value

486 of 0.60 ($R^2_p = 0.57, 0.51, 0.54$ for dry matter, SSC and nitrate content, respectively),
487 they were closed. The equations developed can therefore be taken as an initial
488 approximation to the *in-situ* measurement of quality and safety measurements in intact
489 summer squash using this handheld instrument.

490 For the Matrix-F instrument (prediction set = V2), in the case of the model
491 constructed for predicting dry matter, the SEP(c) does not comply with the protocol
492 while the other statistics lie within the confidence limits (Fig. 3b). SSC fails to meet the
493 validation requirements in terms of R^2_p , while the SEP(c) and bias are below the limits.
494 Additionally, the slope for SSC fails to comply with the values recommended (0.90-
495 1.10). As for the nitrate content, no external validation of the model was carried out due
496 to its limited predictive capacity.

497 In general, the SEP is considered a valuable statistical parameter to evaluate the
498 predictive capacity of an equation, and it is widely accepted that an SEP value of less
499 than 2*SEL shows that the model has an excellent predictive capacity (Westerhaus,
500 1989; Williams, 2001). In this work, the SEL values for dry matter, SSC and nitrate
501 content were 0.30 % fw, 0.07 °Brix and 19.57 mg kg⁻¹, respectively (Table 1). For both
502 instruments, and for the parameter dry matter, the SEP was between 1 and 2, showing
503 the excellent capacity of the NIR model. However, the low SEL values for SSC and
504 nitrate content in comparison with the SEP values obtained for the prediction must be
505 correctly interpreted. It must be considered that the distribution of these components is
506 not homogeneous in the fruit and, whereas the reference values were obtained from the
507 summer squash juice, the spectra were taken from a specific region of the fruit. For this
508 reason, it could be said that a sampling error occurred which was not included in the
509 SEL value. Consequently, the NIRS model developed using both the portable and
510 online instruments for SSC and the nitrate content were marked by their questionable

511 performance, since the SEP value obtained exceeded 5*SEL. Nevertheless, it is
512 important to stress that all the limits and values recommended in the scientific literature
513 and mentioned above refer to other NIRS analysis conditions, i.e. using at-line
514 instruments and using pre-dried and ground samples. In this study, our applications
515 were developed with portable or online devices, using intact samples with a high level
516 of moisture. In this case, the comparison with the limits indicated may be too restrictive.

517 These results suggest the importance of interpreting the SEP values correctly. It
518 is also worth noting how important it is that the NIR spectrophotometers allow both the
519 quality and safety of the product to be guaranteed rapidly and accurately throughout the
520 production chain, from the field to the table, and also permit the number of samples
521 analysed for each batch produced to be increased.

522

523 *3.7. Comparison between the best models developed with the MicroNIRTM 1700 and* 524 *Matrix- F instruments using the C2 calibration set*

525

526 The statistical parameters for the best predictive models developed with the
527 calibration group C2 using the MicroNIRTM 1700 instrument (spectra collection in static
528 mode and a mean spectrum per fruit) and the Matrix-F spectrophotometer (spectra
529 collection in dynamic mode and a mean spectrum per fruit) are shown in Table 5.

530 After the equations were developed for the two instruments, the RPD_{cv} values
531 obtained for each of the parameters tested were compared using Fisher's F test. The
532 predictive capacity of the models developed for the quality parameters with the Matrix-
533 F equipment (RPD_{cv} = 1.98 and RPD_{cv} = 1.63 for dry matter and SSC, respectively) was
534 higher than that obtained with the MicroNIRTM 1700 instrument (RPD_{cv} = 1.72 for dry
535 matter and RPD_c = 1.58 for SSC), and this superiority was significant ($P < 0.05$) in the

536 case of dry matter (Table 5). This greater predictive capacity of the Matrix-F may exist
537 because the equipment is more robust and covers a wider spectral range (834–2502.40
538 nm for Matrix-FT versus 908–1676 nm for MicroNIR™ 1700) with a higher spectral
539 resolution (1.61 nm for Matrix-FT and 6.20 nm for the MicroNIR™ 1700).

540 In the case of the nitrate content, there were no significant differences ($P > 0.05$)
541 in terms of the RPD_{cv} values obtained with the Matrix-F ($RPD_{cv} = 1.36$) and with the
542 MicroNIR™ 1700 ($RPD_{cv} = 1.35$). These results are particularly relevant, since in the
543 case of the MicroNIR™ 1700, it can be seen that in Strategy II (C2 set = 169 fruits) the
544 model's predictive capacity is considerably reduced ($RPD_{cv} = 1.35$) compared with
545 Strategy I (C1 set = 305 fruits; $RPD_{cv} = 1.85$). Since the variability of the calibration
546 group in both cases is similar (Table 1), this lower predictive capacity can be attributed
547 to the smaller number of samples available for developing the models, making them less
548 representative of the different values available over the whole range.

549 These results seem to confirm that, when it comes to measuring the nitrate
550 content with the Matrix-F, the limited predictive capacity obtained could be due to the
551 reduced number of samples available. Thus, if the number of calibration group samples
552 used to develop the model were increased, the model would have been more robust.
553 This is of particular interest to the industry, as this technique could be carried out online
554 as a routine method of analysis, in order to measure not only quality parameters, but
555 also safety parameters such as nitrate content.

556

557 **4. Conclusions**

558

559 The results obtained confirm that NIRS technology can be used as a routine
560 analysis tool to measure quality (dry matter and SSC) in intact summer squashes, both

561 *in-situ* in the field and online in the handling and processing industry, thus enabling to
562 guarantee the quality of the product throughout the whole supply chain. Furthermore,
563 these findings confirm that the two instruments tested would enable to establish
564 adequate uses for summer squashes according to their nitrate content, provided that a
565 sufficiently large and highly representative sample group was available.

566 Additionally, when measuring both quality and safety parameters using the
567 MicroNIR™ 1700, there were no significant differences between the predictive capacity
568 of the models obtained, when the spectra were taken in the fruits *in-situ* in static or
569 dynamic modes. The static mode was therefore selected because its simplicity could
570 enable growers to take the spectra in a faster and simpler way. In the case of the Matrix-
571 F instrument, the results showed that a single spectrum taken while the product passed
572 on the sorting belts would be enough to guarantee the product quality. This would make
573 it extremely easy to use NIRS analysis routinely in the summer squash handling and
574 processing industry.

575 Finally, it must be highlighted that the portable NIR instrument here tested could
576 be a promising tool for its use by the growers during the development of the fruits in the
577 field and at harvest. In addition, NIR technology by means of the incorporation of
578 instruments such as the Matrix-F, could be used to measure the quality and safety of the
579 fruits tested at industry level, in the sorting lines, although it is highly important the
580 optimization of the instrument prior to its final incorporation in the industry.

581

582 **Acknowledgements**

583

584 This research was carried out under the research project ‘Quality determination
585 of summer squash grown in Santaella (Córdoba)’, funded by Gelagri Ibérica, S.L. The

586 authors would like to thank Mrs. María-Carmen Fernández of the Animal Production
587 Department for her technical assistance. The authors would also like to express their
588 gratitude to the Spanish Ministry of Education, Culture and Sports for the support
589 offered to Irina Torres through the Training Programme for Academic Staff (FPU).

590

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675
676

677 **Table 1**

678 Statistical data for the calibration and validation sets selected for each instrument and
 679 standard error of laboratory

Parameter	Statistics	Set 1		Set 2	
		MicroNIR™ 1700		MicroNIR™ 1700 and Matrix-F	
		Training set (C1)	Validation set (V1)	Training set (C2)	Validation set (V2)
Dry matter (% fw)	N ^a	305	107	169	53
	Range	3.22–7.56	3.53–6.94	1.31–7.34	3.67–6.22
	Mean	4.69	4.76	4.93	4.79
	SD ^b	0.74	0.69	0.81	0.71
	CV ^c (%)	15.78	14.50	16.43	14.82
	SEL ^d	0.30			
SSC (°Brix)	N	305	107	169	53
	Range	2.80–6.50	2.80–5.70	2.80–5.63	3.37–5.30
	Mean	4.14	4.16	4.29	4.27
	SD	0.47	0.50	0.49	0.47
	CV (%)	11.35	12.02	11.42	11.01
	SEL	0.07			
Nitrate content (mg kg ⁻¹)	N	305	107	169	53
	Range	18.50–1979.96	20.50–1203.38	23.33–1455.27	39.50–1219.73
	Mean	345.03	375.67	371.02	314.44
	SD	285.37	287.69	309.50	289.13
	CV (%)	82.71	76.58	83.42	91.95
	SEL	19.56			

680 ^a Number of samples.681 ^b Standard deviation.682 ^c Coefficient of variation.683 ^d Standard error of laboratory.

684

685 **Table 2**

686 Calibration statistics for predicting quality and safety parameters in dynamic and static
 687 modes using the MicroNIR™ 1700 instrument (calibration set C1).

Parameter	Mode	N ^a	SECV ^b	R^2_{cv} ^c	RPD _{cv} ^d	F	F _{critical}
Dry matter (% fw)	Dynamic	296	0.42	0.60	1.76	1.102	1.211
	Static	296	0.40	0.62	1.85		
Soluble solid content (°Brix)	Dynamic	293	0.29	0.55	1.62	1.070	1.212
	Static	294	0.30	0.57	1.57		
Nitrate content (mg kg ⁻¹)	Dynamic	293	161.45	0.60	1.77	1.099	1.211
	Static	299	154.01	0.63	1.85		

688 ^a Number of samples.

689 ^b Standard error of cross validation.

690 ^c Coefficient of determination of cross validation.

691 ^d Residual predictive deviation for cross validation.

692

693

694 **Table 3**

695 Comparison between SECV values for the best calibration models obtained using the
 696 Matrix-F and collecting different number of spectra per sample in dynamic mode.
 697 Calibration set C2.

Parameter	SECV ^a 1 spectrum	SECV 2 spectra	SECV 4 spectra	SECV _{min}	SECV _{min} * $\sqrt{F_{critical}}$
Dry matter (% fw)	0.63	0.63	0.58	0.58	0.66
Soluble solid content (°Brix)	0.37	0.35	0.34	0.34	0.39
Nitrate content (mg kg ⁻¹)	297.31	271.03	271.16	271.03	307.84

698 ^a Standard error of cross validation

699

700

701 **Table 4**

702 Calibration statistics for predicting quality and safety parameters using the instruments

703 Matrix-F (dynamic mode and 1 spectrum per fruit). Calibration set C2.

Parameter	N ^a	Range	Mean ^b	SD ^c	SEC ^d	R ² _c ^e	SECV ^f	R ² _{cv} ^g	RPD _{cv} ^h
Dry matter (% fw)	160	3.16–6.85	4.90	0.66	0.32	0.62	0.41	0.62	1.98
Soluble solid content (°Brix)	162	2.88–5.63	4.30	0.45	0.27	0.57	0.30	0.57	1.63
Nitrate content (mg kg ⁻¹)	161	23.30–1077.71	330.31	259.69	216.74	0.25	226.76	0.25	1.36

704 ^a Number of samples.

705 ^b Mean of the calibration set.

706 ^c Standard deviation of the calibration set.

707 ^d Standard error of calibration.

708 ^e Coefficient of determination of calibration.

709 ^f Standard error of cross validation.

710 ^g Coefficient of determination of cross validation.

711 ^h Residual predictive deviation for cross validation.

712

713

714 **Table 5**

715 Calibration statistics for predicting quality and safety parameters using the instruments

716 Matrix-F (dynamic mode and 1 spectrum per fruit) and MicroNIR™ 1700 (static mode

717 and 1 spectrum per fruit). Calibration set C2

718

Parameter	Instrument	N ^a	SEC ^b	R ² _c	SECV ^d	R ² _{cv} ^e	RPD _{cv} ^f	F	F _{critical}
Dry matter (%) fw)	Matrix F	160	0.32	0.62	0.41	0.62	1.98	1.31	1.299*
	MicroNIR™ 1700	161	0.44	0.58	0.47	0.58	1.72		
Soluble solid content (°Brix)	Matrix-F	162	0.27	0.57	0.30	0.57	1.63	1.07	1.296
	MicroNIR™ 1700	164	0.28	0.57	0.31	0.57	1.58		
Nitrate content (mg kg ⁻¹)	Matrix-F	161	216.74	0.25	226.76	0.25	1.36	1.02	1.299
	MicroNIR™ 1700	159	221.42	0.23	228.64	0.23	1.35		

719 * Significant differences ($P < 0.05$).

720 ^a Number of samples.

721 ^b Standard error of calibration.

722 ^c Coefficient of determination of calibration.

723 ^d Standard error of cross validation.

724 ^e Coefficient of determination of cross validation.

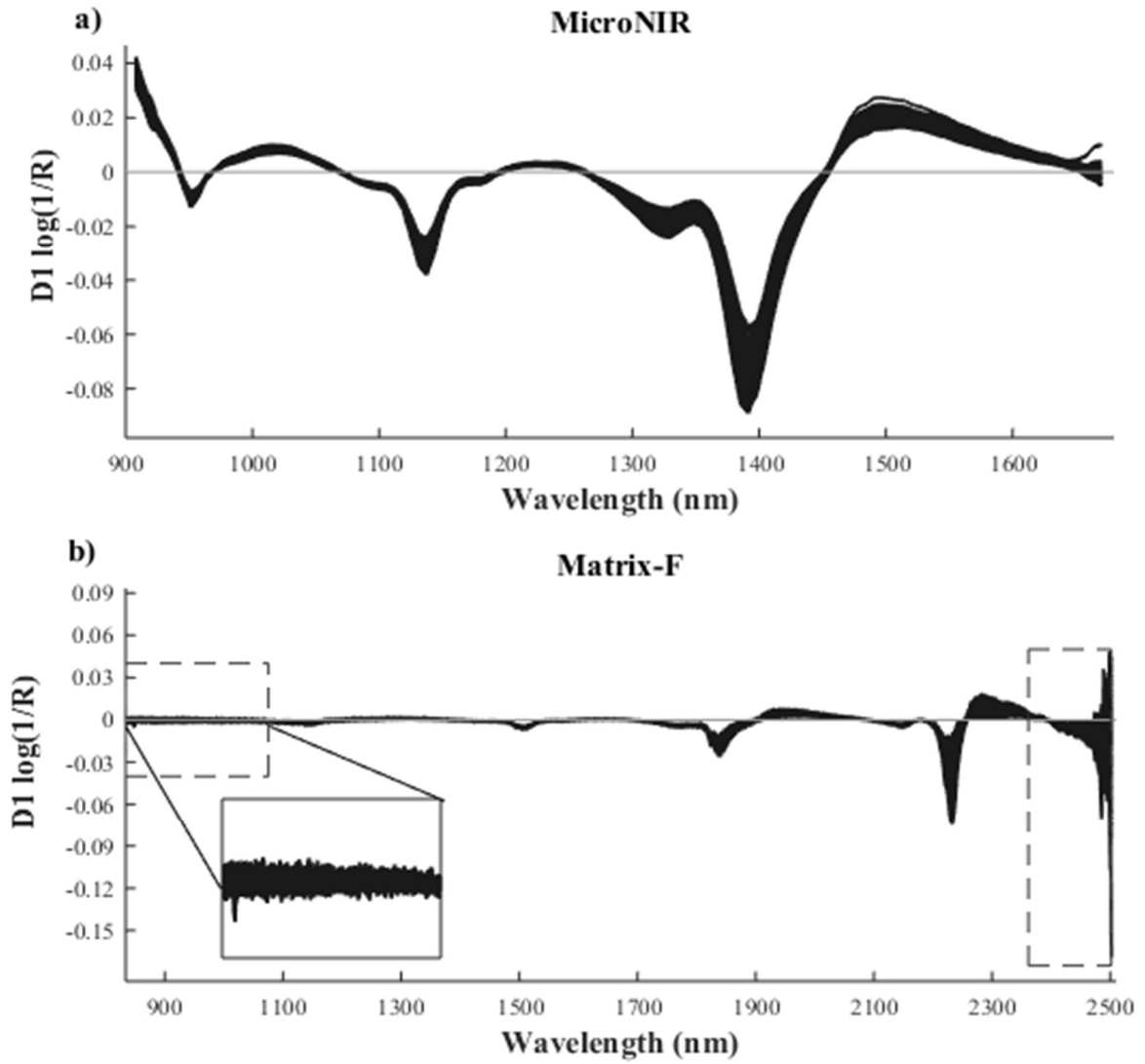
725 ^f Residual predictive deviation for cross validation.

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728

729 **Fig. 1.** First derivative spectra of summer squashes prior to removing the noise using
730 the MicroNIR™ 1700 (a) and Matrix-F (b).

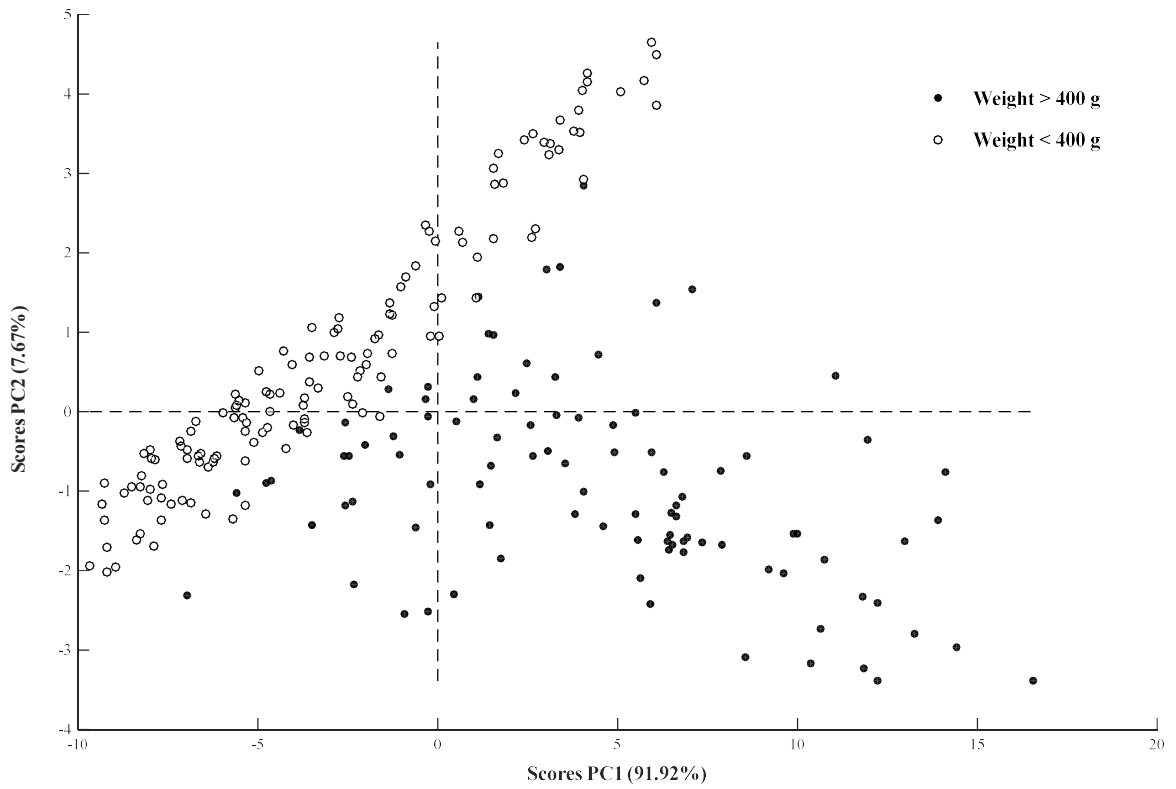


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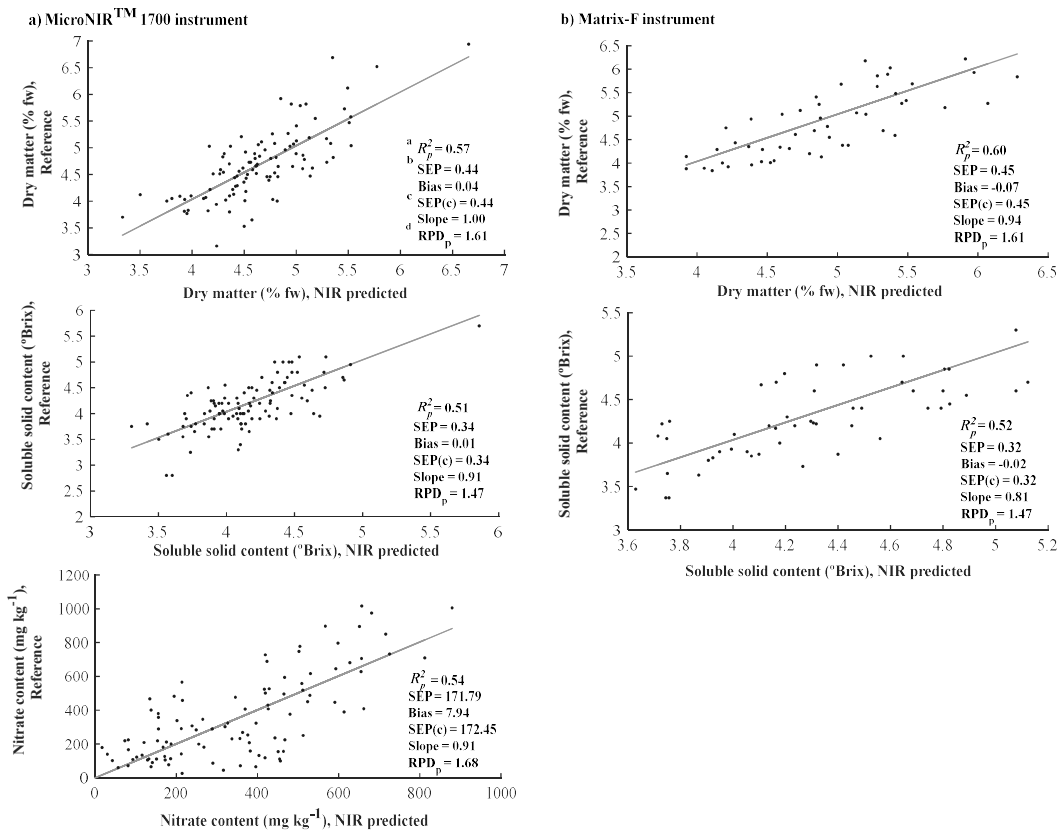
732

733 **Fig. 2.** Scores plot for the first (PC1) and second (PC2) principal components for
734 summer squashes using the Matrix-F instrument.

735



736 **Fig. 3.** Reference and NIR predicted values for quality and safety parameters using the
 737 MicroNIR™ 1700 (a) and Matrix-F (b) instruments.



738

739 ^a Coefficient of determination of prediction.

740 ^b Standard error of prediction.

741 ^c Standard error of prediction bias-corrected.

742 ^d Residual predictive deviation for prediction.