

1 **Assessment of macadamia nut quality defects by means of near infrared**
2 **spectroscopy (NIRS) and nuclear magnetic resonance (NMR)**

3

4 Lívia Cirino de Carvalho^a, Fabíola Manhas Verbi Pereira^b, Camilo de Lelis Medeiros
5 de Moraes^c, Kássio Michell Gomes de Lima^d, Gustavo Henrique de Almeida Teixeira^{e,*}

6

7 ^aUniversidade Estadual Paulista (UNESP), Faculdade de Ciências Farmacêuticas
8 (FCFAR), Departamento de Alimentos e Nutrição, Campus de Araraquara, Rodovia
9 Araraquara-Jaú, km 1 – CP 502, CEP: 14.800-903, Araraquara, São Paulo, Brazil.

10 ^bUniversidade Estadual Paulista (UNESP), Instituto de Química (IQ), Campus de
11 Araraquara. Rua Prof. Francisco Degni, 55, CEP: 14800-060, Araraquara – SP, Brazil.

12 ^cUniversity of Central Lancashire, School of Pharmacy and Biomedical Sciences,
13 Preston, Lancashire, PR1 2HE, United Kingdom.

14 ^dUniversidade Federal do Rio Grande do Norte (UFRN), Instituto de Química, Química
15 Biológica e Quimiometria, Avenida Senador Salgado Filho, n° 3000, Bairro de Lagoa
16 Nova, CEP: 59.078-970, Natal, Rio Grande do Norte, Brazil.

17 ^eUniversidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e
18 Veterinárias (FCAV), Campus de Jaboticabal. Via de Acesso Prof. Paulo Donato
19 Castellane s/n, CEP: 14.884-900, Jaboticabal, São Paulo, Brazil.

20 *Corresponding author: gustavo@fcav.unesp.br

21

22

23 **Abstract**

24 Macadamia kernels are visually sorted based on the presence of quality defects
25 by specialized labors. However, this process is not as accurate as non-destructive
26 methods such as near infrared spectroscopy (NIRS) and nuclear magnetic resonance
27 (NMR). Thus, NIRS and NMR in combination with chemometrics have become
28 established non-destructive method for rapid assessment of quality parameters in the
29 food and agricultural sectors. Therefore, the quality of macadamia nuts was assessed by
30 NIRS and NMR using chemometric tools such as PCA-LDA and GA-LDA to evaluate
31 kernel defects. Macadamia kernels were classified as: 1=good, marketable kernels
32 without defects; 2=kernels with discoloration; 3=immature kernels; 4=kernels affected
33 by mold; and 5=kernels with insect damage. Using NIRS, the GA-LDA resulted in an
34 accuracy and specificity of 97.8 % and 100 %, respectively, to classify good kernels. On
35 the other hand, PCA-LDA technique resulting in an accuracy higher than 68 % and
36 specificity of 97.2 % to classify immature kernels. For NMR, PCA-LDA resulted in an
37 accuracy higher than 83% and GA-LDA resulted in an accuracy of 100%, both to
38 classify kernels with insect damage. NIRS and NMR spectroscopy can be successfully
39 used to classify unshelled macadamia nuts based on the defects. However, NIRS out-
40 performed NMR based on the higher accuracy results.

41

42 **Keywords:** *Macadamia integrifolia* Maiden & Betche, TD-NMR, PCA-LDA, GA-
43 LDA, chemometrics.

44

45

46 **1. Introduction**

47 Macadamia (*Macadamia integrifolia* Maiden & Betche) nut growers are keen
48 to continuously improve nut quality as this is the main characteristic required by the
49 final consumers. Nogueira (2008) mentioned that the quality of macadamia fruit is
50 associated with favorable climatic conditions, planning and orchard management,
51 varieties, pest control, plant nutrition, harvest and post-harvest practices. All these
52 factors are decisive for macadamia development and nut quality.

53 According to O'Hare et al. (2004), the main defects that can be observed in
54 macadamia nuts are immaturity; small nuts; cracks in the shell that allow the occurrence
55 of biological and chemical contamination; lipids oxidation, which result in unpleasant
56 odor and taste; bruises, and high moisture. Guthrie et al. (2004) reported other defects
57 that may be considered, as such: fungal growth, decomposition, germination, and
58 discoloration of macadamia nuts. Therefore, sound and/or good macadamia nuts must
59 have light cream color, no signs of mold, decay, insect scars, blemishes, hollow centers,
60 dark centers, shriveling, off-odors, adhering shells, and loose of extraneous material
61 (Wall, 2013).

62 Macadamia industry has developed various parameters of quality standards.
63 The Southern African Macadamia Growers' Association (SAMAC) classifies
64 macadamia nuts into three classes: first grade, commercial grade, and local market.
65 These classes are established based on kernel color, flavor and odor, kernel dust, insect
66 infestation, foreign material. A limit of 1.5 % is used reject the nuts based on the
67 presence of insect damage, discoloration, and immaturity (SAMAC, 2018). On the other
68 hand, the United Nations Economic Commission for Europe (UNECE) has a higher
69 tolerance (5 %) for the presence of these defects (UNECE, 2010).

70 The sorting process of macadamia kernels in the industry can be carried out
71 manually (Piza, 2005) or electronically (France, 2007), but both present flaws, since
72 manual sorting of defective kernels can decrease dramatically with the use of inadequate
73 lighting and untrained personnel, and the electronic selection uses color to sort kernels,
74 which may lead to improper selection, since immature kernels can only be identified
75 based on the deformed, wrinkled, and shrunken kernel (SAMAC, 2018).

76 The increasing requirements of consumers, regulatory agencies, and
77 competitors have been an impulse for the development of more accurate quality
78 assessment techniques in the food industry. In this regard, near infrared spectroscopy
79 (NIRS) in combination with chemometric modelling have become an established
80 method for rapid assessment and non-destructive quality parameters in the food and
81 agricultural sectors (Abbott, 1999; Jensen et al., 2001), since it is fast, safe, relatively
82 inexpensive technique and provides automation of quality control processes in products
83 of agroindustry (Pasquini, 2003).

84 NIRS has been used to evaluate macadamia nut quality. Guthrie et al. (2004)
85 developed modified partial least squares regression (MPLS) models for oil content
86 determination in intact macadamia kernels with a root mean square error of calibration
87 (RMSEC) of 2.4 % and discriminated intact kernels with brown centers or rancidity
88 from each other and from sound kernels using PCA. Canneddu et al. (2016) developed
89 models for predicting peroxide value (PV) and acidity index (AI) using PLSR and
90 classification models to discriminate defects present on shelled macadamia nuts using
91 FT-NIR. The best model for PV prediction resulted in a coefficient of determination
92 (R_p^2) of 0.72, and for AI prediction a SEP of 0.14 % and a R_p^2 of 0.80. Adequate
93 classification models (93.2 %) for defects was possible using principal component
94 analysis linear discriminant analysis (PCA-LDA). Carvalho et al. (2017) classified

95 intact macadamia nuts according to cultivars using PCA-LDA and genetic algorithm
96 with linear discriminant analysis (GA-LDA), reporting an accuracy higher than 94.4 %
97 and a value of 82.7 % for sensitivity using GA-LDA, respectively. The better
98 performance of GA-LDA can be due to that GA algorithm selects several wavenumbers
99 in a single band, due to collinearity problems. Carvalho et al. (2019) evaluated the
100 oxidative stability in intact macadamia nuts during drying process and reported a SEP
101 of 0.55 meq.kg⁻¹ and R²c of 0.57 for PV prediction, and SEP of 0.14 % and R²c of 0.29
102 for AI prediction. These results demonstrate that NIRS can be used to assess the
103 oxidative stability of intact macadamia nuts.

104 Nuclear magnetic resonance (NMR) has also been stated as an alternative
105 method among non-destructive techniques to evaluate fruit quality (Abbott, 1999). TD-
106 NMR has wide applications for qualitative and quantitative in food analysis (Conalgo,
107 1996). In this regard, Pedersen et al. (2000) combined low-field nuclear magnetic
108 resonance (LF-NMR) and PCA to classify rape and mustard seeds according on the
109 type of seed, obtaining two distinct groups and 100 % of explained variance. This
110 technique was also applied to evaluate the efficacy of hydrophobic coatings as a barrier
111 to the oxidation of macadamia nuts (Colzato et al., 2009).

112 Although some results can be found regarding the use of NIRS to assess
113 macadamia quality defects (Canneddu et al., 2016), this study was performed evaluating
114 the macadamia in nut not the kernel (unshelled), and no reports were found on using
115 NMR to evaluate macadamia kernel defects. Therefore, the objective of this study was
116 to develop NIRS and NMR calibration models to evaluate macadamia kernels based on
117 the most common defects aiming to improve the quality control process in the
118 macadamia industry.

119

120 **2. Material and Methods**

121 **2.1. Plant material**

122 *Macadamia* (*Macadamia integrifolia* Maiden & Betche) kernels were obtained
123 in a commercial orchard located in Dois Córregos, São Paulo, Brazil (22° 37' S, latitude,
124 48° 38' W, longitude, 753 m altitude) in 2017 harvest season. Nuts were harvested three
125 times during the season (April, June, and August) and kernels were visually sorted by
126 the industry personnel based on their quality attributes, as such: 1=good, marketable
127 kernels without defects; 2=kernels with discoloration; 3=immature kernels; 4=kernels
128 affected by mold; and 5=kernels with insect damage. (Figure 1). These quality attributes
129 represented the five studied classes (model). It is important to state that the nuts were
130 dried by the processing industry and used in the analyses without any previous
131 treatment.

132 **2.2. NIR spectra acquisition**

133 On the surface of each macadamia kernel two Fourier Transformed (FT) NIR
134 reflectance spectra (11,544 – 3,952 cm^{-1} , nm, resolution of 16 cm^{-1} , and 64 scans) were
135 collected using a Bruker NIR spectrometer (Tango, Ettlingen, Germany) after
136 temperature stabilization at $\sim 25^\circ\text{C}$. The two replica spectra measured per nut were
137 averaged, so the model is made on a sample basis. Samples were collected in three
138 different harvests, where 20 nuts were sorted and used for spectra acquisition for each
139 defect class. This resulted in a total of 300 measured samples (20 nuts x 5 classes x 3
140 harvests).

141 **2.3. Time domain (TD) NMR measurements**

142 TD-NMR measurements of macadamia kernels (n=100) were carried out at 22
143 $^\circ\text{C}$ in a 0.27 T (11.3 MHz for ^1H) benchtop SLK200 Spinlock instrument (Spinlock
144 Magnetic Resonance Solutions, Cordoba, Argentina). The measurements were

145 performed using the standard CPMG sequence to obtain the exponential decay signal
146 that is governed by the transverse relaxation time (T_2). The sequence used $\pi/2$ and π of
147 11.6 and 19.6 μs , respectively, an echo time of 600 μs , 4 scans and 1500 echoes.
148 Samples harvested in June 2017 were used and for each defect class 20 nuts were sorted
149 and used for spectra acquisition, totaling 100 spectra. The mass of the samples ranged
150 from 14 to 24 g depending on the sample density. The samples were the same used to
151 collect the NIRS spectra, but the spectra were collected on different days.

152 **2.4. Chemometrics**

153 Data analysis of NIR and TD-NMR were performed within MATLAB R2014b
154 environment (MathWorks Inc., USA) using PLS Toolbox version 7.9.3 (Eigenvector
155 Research Inc., USA) and lab-made routines. Three different pre-processing methods
156 were applied to test the averaged sample spectrum (average of 10 spectra per sample):
157 (1) only mean-centering; (2) standard normal variate (SNV) followed by mean-
158 centering; (3) Savitzky-Golay second derivative (window of 5 points, 2nd order
159 polynomial function) followed by mean-centering. The data was split into training (70
160 %, 210 samples), validation (15 %, 45 samples) and test (15 %, 45 samples) sets using
161 the Kennard-Stone sample selection algorithm (Kennard and Stone, 2012). The training
162 and validation sets were used for model construction and internal optimization,
163 respectively; while the test set was used to evaluate the final predictive performance of
164 the classification models built towards external samples.

165 Multivariate classification was performed by means of principal component
166 analysis linear discriminant analysis (PCA-LDA) and genetic algorithm linear
167 discriminant analysis (GA-LDA). PCA-LDA performs a feature extraction using
168 principal component analysis (PCA) followed by a linear discriminant classifier (LDA)
169 (Morais and Lima, 2018) For this, PCA is applied to the pre-processed data reducing

170 the original number of variables (i.e., wavelengths) to a few number of principal
 171 components (PCs) accounting for the majority of the original data variance. Each PC is
 172 composed by scores and loadings, where the first represents the variance between the
 173 samples and the latter the variance on wavelength direction (Bro and Smilde, 2014).
 174 LDA is applied to the PCA scores in a non-Bayesian form as follows (Dixon and
 175 Brereton, 2009; Wu et al, 1996).

$$176 \quad L(\mathbf{x}_i) = (\mathbf{x}_i - \bar{\mathbf{x}}_k)^T \mathbf{C}_{\text{pooled}}^{-1} (\mathbf{x}_i - \bar{\mathbf{x}}_k) \quad (1)$$

177 where $L(\mathbf{x}_i)$ represents the LDA classification scores for sample i ; \mathbf{x}_i is the input vector
 178 (i.e., the PCA scores) for sample i ; $\bar{\mathbf{x}}_k$ is the average vector of class k ; $\mathbf{C}_{\text{pooled}}$ is pooled
 179 covariance matrix; and T represents the matrix transpose operation.

180 GA-LDA is feature selection technique followed by an LDA classifier. Initially,
 181 a genetic algorithm (GA) is applied to reduce to the spectral data into a few number of
 182 variables based on an evolutionary process (Bro and Smilde, 2014); then LDA is
 183 applied to these variables according to Eq. 1. These variables are in the same scale of
 184 the original spectral data and are selected according to the lowest risk of miss
 185 classification G . G is calculated in the validation set as (Carvalho et al. 2017).

$$186 \quad G = \frac{1}{N_v} \sum_{n=1}^{N_v} g_n \quad (2)$$

187 where N_v is the number of validation samples and g_n is defined as:

$$188 \quad g_n = \frac{r^2(x_n, m_{I(n)})}{\min_{I(m) \neq I(n)} r^2(x_n, m_{I(m)})} \quad (3)$$

189 in which the numerator is the squared Mahalanobis distance between sample x_n (of
 190 class index $I(n)$) and the mean $m_{I(n)}$ of its true class; and the denominator represents
 191 the squared Mahalanobis distance between sample x_n and the mean $m_{I(m)}$ of the
 192 closest wrong class. GA was performed through 100 generations, having 200
 193 chromosomes each. Cross-over and mutation probabilities were set at 60% and 1%,
 194 respectively. The algorithm was repeated three times and the best result was chosen.

195

196 **2.5. Figures of merit**

197 The classification performance of each algorithm was evaluated according to
 198 the quality parameters of accuracy (total number of samples correctly classified
 199 considering true and false negatives), sensitivity (proportion of positives correctly
 200 identified) and specificity (proportion of negatives correctly identified). These
 201 parameters are calculated as follows (Morais and Lima, 2017):

$$202 \quad \text{Accuracy (\%)} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{FP} + \text{TN} + \text{FN}} \times 100 \quad (4)$$

$$203 \quad \text{Sensitivity (\%)} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100 \quad (5)$$

$$204 \quad \text{Specificity (\%)} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100 \quad (6)$$

205 where TP stands for true positives; TN for true negatives; FP for false positives; and FN
 206 for false negatives.

207

208 **3. Results and Discussion**

209 3.1. NIR spectra

210 The raw FT-NIR spectra obtained from all macadamia kernels and the average
211 spectra from each quality attribute class can be seen in Figure 2. It was not possible to
212 observe spectral differences between the quality attributes when all macadamia kernels
213 were assessed (Figure 2A). On the other hand, the mean spectra were quite different for
214 each defect category (Figure 2B), especially at the wavelength 1,900 nm to 2,500 nm.

215 The FT-NIR spectra presented absorption bands at 1,200 nm, which are related
216 to CH stretch second overtone (Cozzolino et al., 2005), while those at 1,700 – 1,800 nm
217 are associated to the first overtones of CH stretching vibrations of $-\text{CH}_3$, $-\text{CH}_2-$ and $-\text{HC}=\text{CH}$ (Armenta and La Guardia, 2007). Absorption bands at 1,350 – 1,600 nm and
218 1,950 nm and 2,100 nm are related to the presence of glucose, sucrose, and fructose
219 (Lanza and Li, 1984) and immature kernels have higher sucrose and reducing sugar
220 contents than fully mature kernels (Wall, 2013). In Figure 2B can be seen that at 1,350
221 – 1,600 nm the immature kernels exhibit a higher absorption intensity, since maturity is
222 inversely related to sugar content (Ripperton et al., 1938).

224 The wavelength region situated at 2,200 – 2,500 nm is mainly related to the
225 oxidation and hydrolytic degradation of lipids (Cozzolino et al. 2005). It is possible to
226 observe that the immature kernels, classified as kernel which is misshapen, abnormally
227 small or partially aborted, including shriveled and shrunken kernels (SAMAC, 2016)
228 present a lower absorption band (2,200 nm - 2,500 nm) (Figure 2B). This result might
229 be due to the fact that maturity is correlated with oil content (Cavaletto, 1985),
230 consequently with less lipid degradation.

231 3.1.1. Model development

232 To correlate the FT-NIR spectra to the quality categories, discriminant
233 classifications based on PLS-DA and GA-LDA were used and compared and evaluated
234 in terms of sensitivity, specificity, accuracy, separately for each category.

235 Regarding pre-processing, SNV lead to best results using PCA-LDA, resulting
236 in an accuracy of 68 % and a specificity of 97 % for immature kernels (Table 1). The
237 accuracy shows the proportion of samples correctly grouped, while specificity
238 represents the probability of a sample without the desired characteristic to be given a
239 negative test result (Amodio et al., 2017). However, the sensitivity presented low
240 values (67 %), and this parameter describes the model ability to correctly recognize
241 samples belonging to a class (Ballabio and Consonni, 2013). For example, if none of
242 the marketable kernels were classified as other class (FN is equal to zero), the
243 sensitivity for the marketable kernels class would have been equal to 100 %.

244 Cannedu et al. (2016) classified marketable macadamia kernels in relation to
245 non-marketable kernels using PLS-DA and reported percentages of 88 % for calibration
246 and 87 % for prediction. These results were inferior than what we obtained, probably
247 because we used more samples ($n = 300$) than Cannedu et al. (2016) ($n = 100$).
248 Therefore, the inclusion of more data into the dataset improved the robustness and
249 increase the classification accuracy.

250 Marketable kernels and kernels with defects (immature, insect damage, mold,
251 and discoloration) could be discriminated from each other using GA-LDA (Figure 3).
252 The accuracy and specificity of GA-LDA for marketable kernels achieved a value of
253 97.8 % and 100 %, respectively (Table 2).

254 To perform the GA-LDA, some of the wavelengths were selected (Table 3).
255 This selection was based on compounds of particular interest, e.g., 1,020 nm and 1,173
256 nm, representing the C–H groups from lipids; 1,485 nm and 1,789 nm, related to the

257 first overtone of stretching and anti-symmetric O–H bond and second overtone of
258 stretching O–H bend, respectively. Absorption bands at the wavelength near 1,450 and
259 1,940 nm are related to the presence of water in foods (Moscetti et al., 2014) and this
260 explains why the wavelengths 1,485 nm, 1,975 nm and 1,987 nm were selected by GA.

261 It is possible to observe that the kernels with discoloration had a higher
262 moisture content than the others (Figure 2B), and these moisture contents correspond to
263 water activities (a_w) greater than 0.8 at which browning reaction rates are high (Wall,
264 2013), and maintaining nuts-in-shell at high moisture content can cause discoloration
265 (Walton et al., 2013).

266 **3.2. TD-NMR**

267 The typical curves of the CPMG decays for the different defects found in
268 macadamia kernels can be seen in Figure 4. It can be observed that kernels with insect
269 damage presented a faster settling time compared to the others, whereas the kernels with
270 presence of fungi (moldy) showed the slower signal decay (Figure 4).

271 The intensity of the TD-NMR signals from relaxation (our case) and diffusion
272 measurements is related to the water content related to water status, water
273 compartmentalization and molecular mobility in the food sample (Kirtil et al., 2017). In
274 order to evaluate the influence of the water content on the nutrient content of the food, it
275 is important to note that there are variations in the moisture content of the kernels, since
276 these moisture contents correspond to water activities at which microbial growth rates
277 are high (Wall, 2013). This explains the fact that moldy kernels have a higher moisture
278 content.

279 In Figure 5 it is possible to observe that there was not a clear separation
280 between the defect classes. However, in Figure 5A there was a tendency of separation
281 between the good and immature kernels. Probably because there are differences in the

282 decay time between these classes (Figure 4), with showed that the most rapid decay is
283 due to solid components, mainly composed of proteins and carbohydrates (Prestes et al.,
284 2007) and immature kernels present a higher carbohydrate concentration, represented by
285 sucrose and fructose higher than mature kernels (Wall, 2013).

286 *3.2.1. Model development*

287 The best TD-NMR classification models were obtained using the PCA-LDA and
288 GA-LDA without pre-processing the signals (Table 4). Using PCA-LDA, it was
289 possible to achieve 86 % accuracy for the training set and 83.3 % for the validation set
290 to classify kernels with insect damage. On the other hand, the GA-LDA analysis
291 obtained 64 % for the calibration set and 100 % for the validation set, allowing the use
292 of this model to classify kernels with insect damages.

293 TD-NMR has been used to classify other oleaginous produces including nuts.
294 Di Caro et al. (2017) studying not damaged and moldy hazelnuts kernels highlighted
295 that NMR might be used to discriminate oils extracted from both kernel classes. Di Caro
296 (2018) also reported that using NMR was possible to obtain values of 97 % for
297 sensitivity and 81 % for specificity to classify in-shell damaged hazelnuts. Therefore,
298 NMR might be a useful analytical tool for quality control in nut industry.

299 **3.3. NIRS versus TD-NMR**

300 The results obtained from both techniques for the development of the
301 classification models for macadamia kernels quality defects can be seen in Table 1, 2,
302 and 4. Overall, the NIRS showed better classification capability as higher values of
303 accuracy were obtained using GA-LDA models. The lower performance of the
304 classification models developed using the TD-NMR signals might be related to the
305 number of samples, as just the kernels harvested in June 2017 were used.

306 NIRS and TD-NMR present many similarities as they are fast non-destructive
307 analytical methods, do not need sophisticated sample preparation, and the results can be
308 collected, processed, and stored directly in a microcomputer (Colnago, 1996; Pasquini,
309 2003). However, when it comes to NMR spectroscopy, high cost is normally considered
310 as one of the most serious drawbacks and this technique requires special skills to
311 interpret the spectra acquisition (Xu et al., 2015). Another limitation of NMR
312 spectroscopy is the insensitivity to minor fat component detection (Kucha et al., 2018).
313 These suggest that, due the fact that NIRS is useful for detecting components with up to
314 0.1 % concentration (Xu et al., 2015) and NMR presents lower sensitivity, NIRS models
315 presented more satisfactory results.

316

317 **4. Conclusions**

318 NIRS and TD-NMR combined with chemometric methods proved to be
319 powerful tools to classify macadamia kernels based on their quality defects. However,
320 NIRS out-performed TD-NMR based on the higher accuracy results.

321 NIRS and TD-NMR spectroscopy can be successfully used to evaluate the
322 quality of unshelled macadamia nuts and have potential to improve the existing
323 postharvest techniques used in the macadamia industry.

324

325 **Acknowledgment**

326 This study was financed in part by the Coordenação de Aperfeiçoamento de
327 Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

328

329 **References**

330 Abbott, J.A. (1999). Quality measurement of fruit and vegetables. *Postharvest Biology*
331 *and Technology*, 15, 207-223.

- 332 Amodio, M.L., Ceglie, F., Chaudhry, M.M., Piazzolla, F. & Colelli, G. (2017).
333 Potential of NIR spectroscopy for predicting internal quality and discriminating among
334 strawberry fruits from different production systems. *Postharvest Biology and*
335 *Technology*, 125, 112– 121. Doi.Org/10.1016/J.Postharvbio.2016.11.013.
- 336 Armenta, S., Garrigues, S. & De la Guardia, M. (2007). Determination of edible oil
337 parameters by near infrared spectrometry. *Analytica Chimica Acta*, 596, 330-337.
338 Doi:10.1016/j.aca.2007.06.028
- 339 Ballabio, D., & Consonni, V. (2013). Classification tools in chemistry. Part 1: linear
340 models. PLS-DA. *Analytical Methods*, 5, 3790–3798. Doi: 10.1039/c3ay40582f
- 341 Bro, R., & Smilde A. K. (2014). Principal component analysis. *Analytical Methods*, 6,
342 2812-2831. Doi:10.1039/c3ay41907j
- 343 Canneddu, G., Júnior, L.C. & Teixeira, G.H.A. (2016). Quality evaluation of shelled
344 and unshelled macadamia nuts by means of near-infrared spectroscopy (NIR). *Journal*
345 *of Food Science*, 81, 1613-1621. Doi.org/10.1111/1750-3841.13343
- 346 Carvalho, L.C., Morais, C.L., Lima, K.M.G., Cunha Júnior, L.C., Nascimento, P.A.,
347 Faria, J.B. & Teixeira, G.H.A. (2016). Determination of the geographical origin and
348 ethanol content of Brazilian sugarcane spirit using near-infrared spectroscopy coupled
349 with discriminant analysis. *Analytical Methods*, 8, 5658-5666.
- 350 Carvalho, L.C., Morais, C.L.M, Lima, K.M.G., Leite, G.W.P., Oliveira, G.S.,
351 Casagrande, I.P., Santos Neto, J.P. & Teixeira, G.H.A. (2018). Using intact nuts and
352 near infrared spectroscopy to classify macadamia cultivars. *Food Analytical Methods*,
353 11, 1857–1866. <https://doi.org/10.1007/s12161-017-1078-9>
- 354 Carvalho, L.C., Marcondes, L.L., Morais, C.L.M., Lima, K.M.G., & Teixeira, G.H.A.
355 (2019). Non-destructive assessment of the oxidative stability of intact macadamia nuts
356 during the drying process by near-infrared spectroscopy. *LWT – Food Science and*
357 *Technology*, 103, 101-107. Doi.org/10.1016/j.lwt.2018.12.056
- 358 Colnago, L.A. (1996). *Análise do teor de óleos em sementes por RMN*. São Carlos:
359 Embrapa Instrumentação Agropecuária. Circular Técnica, 3, 14p.

- 360 Cozzolino, D., Murray, I., Chree, A. & Scaife, J.R. (2005). Multivariate determination
361 of free fatty acids and moisture in fish oils by partial least-squares regression and near-
362 infrared spectroscopy. *LWT – Food Science and Technology*, 38, 821-828.
- 363 Di Caro, D. (2018). NMR measurements for hazelnuts classification. PhD dissertation.
364 Università Degli Studi Di Salerno. Salerno, Italy.
- 365 Liguori, C., Pietrosanto, A., Sommella, P. (2017). Hazelnut oil classification by NMR
366 techniques. *IEEE transactions on instrumentation and measurement*, 66, 928-934. Doi:
367 10.1109/TIM.2016.2640438
- 368 Di Caro, D., Liguori, C., Pietrosanto, A., Sommella, P. (2017). Hazelnut oil
369 classification by NMR techniques. *IEEE transactions on instrumentation and
370 measurement*, 66, 928-934. Doi: 10.1109/TIM.2016.2640438
- 371 Dixon, S.J., & Brereton, R.G. (2009). Comparison of performance of five common
372 classifiers represented as boundary methods: Euclidean distance to centroids, linear
373 discriminant analysis, quadratic discriminant analysis, learning vector quantization and
374 support vector machines, as dependent on data structure. *Chemometrics and Intelligent
375 Laboratory Systems*, 95, 1-17.
- 376 Jensen, P.N., Sorensen, G., Soren, B.E. & Bertelsen, G. (2001). Evaluation of quality
377 changes in walnut kernels (*Juglans regia* L.) by Vis/NIR spectroscopy. *Journal of
378 Agricultural and Food Chemistry*, 49, 5790–5796. Doi: 10.1021/jf010509t
- 379 Kennard, R.W., & Stone, L.A. (1969). Computer aided design of experiments.
380 *Technometrics*, 11, 137–148. Doi: 0.1080/00401706.1969.10490666
- 381 Kirtil, E., Cikrikci, S., Mccarthy, M. J., & Oztop, M.H. (2017). Recent advances in time
382 domain NMR & MRI sensors and their food applications. *Current Opinion in Food
383 Science*, 17, 9-15.
- 384 Kucha, C.T., Liu, L., & Ngadi, M.O. (2018). Non-destructive spectroscopic techniques
385 and multivariate analysis for assessment of fat quality in pork and pork products: a
386 review. *Sensors*, 18, 1-23.

- 387 Lanza, E. & Li, B.W. (1984). Applications of near infrared spectroscopy for detecting
388 the sugar content of fruit juices. *Journal of Food Science*, 49, 995-998.
- 389 McCall, J. (2005). Genetic algorithms for modelling and optimization. *Journal of*
390 *Computational and Applied Mathematics – Special Issue: Mathematics applied to*
391 *immunology*, 184, 205-222.
- 392 Medeiros, E. P., Feliz, P.H.D., Melo, J.K.A., Barreto, A. N., & Nascimento, J.J.V.R.
393 (2008). Um método não destrutivo por RMN de baixo campo e quimiometria para
394 classificação de sementes de mamona em função do perfil espectral do óleo. III
395 Congresso brasileiro de Mamona, energia e recinoquímica.
- 396 Morais, C.L.M. & Lima, K.M.G. (2017). Comparing unfolded and two-dimensional
397 discriminant analysis and support vector machines for classification of EEM data.
398 *Chemometrics and Intelligent Laboratory Systems*, 170, 1-12
- 399 Morais, C.L.M. & Lima, K. M. G. (2018) Principal component analysis with linear and
400 quadratic discriminant analysis for identification of cancer samples based on mass
401 spectrometry. *Journal of the Brazilian Chemistry Society*, 29, 472-481.
- 402 Nogueira, N.R. (2008). O agronegócio da macadâmia: uma breve análise dos aspectos
403 de produção, econômico e social. *Interface tecnológica*, 5, 21-28.
- 404 Pasquini, C. (2003). Near Infrared Spectroscopy: Fundamentals, Practical Aspects and
405 Analytical Applications. *Journal of Brazilian Chemistry Society*, 14, 198-219.
- 406 Pedersen, H., Munck, L., & Engelsen, S. (2000). Low-field ¹H nuclear magnetic
407 resonance and chemometrics combined for simultaneous determination of water, oil,
408 and protein contents in oilseeds. *Journal of the American Oil Chemists' Society*, 77,
409 1069–1077.
- 410 Ripperton, J.C., Moltzau, R.H., & Edwards, D.W. (1938). *Methods of evaluating the*
411 *macadamia nut for commercial use and the variation occurring among seedling*
412 *plantings in Hawaii*. Washington: Hawaii Agricultural Experiment Station Bulletin, N°.
413 79.

- 414 SAMAC. Southern African Macadamia Growers' Association. (2018). Raw macadamia
415 products quality specifications. <http://www.samac.org.za/index.php/standards/> Accessed
416 12 August 2018.
- 417 O'Hare, P., Hidden, D.V., Burton, D. & Salmon, T. (2004). Australia macadamia
418 industry code of sound orchard practices. Lismore: Australian macadamia society.
- 419 Xu, J., Riccioli, C., & Sun D. (2015). An overview on destructive spectroscopic
420 techniques for lipid and lipid oxidation analysis in fish and fish products.
421 *Comprehensive Reviews in Food Science and Food Safety*, 14, 466-477.
- 422 Wall, M.M. (2013). Improving the quality and safety of macadamia nuts. In L.J. Harris,
423 (Ed.), *Improving the quality and safety of nuts* (pp.274-296). Cambridge: Woodhead
424 Publishing Series in Food Science and Technology and Nutrition.
- 425 Walton, D.A., Randall, B.W., Le Lagade, M.D. & Wallace, H.M. (2013). Maintaining
426 high moisture content of macadamia nuts-in-shell during storage induces brown centres
427 in raw kernels. *Journal of the Science of Food and Agriculture*, 93, 2953–295.
- 428 Wu, W., Mallet, Y., Walczak, B., Penninckx, W., Massart, D.L., Heuerding, S. & Erni,
429 F. (1996). Comparison of regularized discriminant analysis linear discriminant analysis
430 and quadratic discriminant analysis applied to NIR data. *Analytica Chimica Acta*, 329,
431 254-265.
- 432

433

434 **Tables**435 **Table 1.** Values of accuracy, sensitivity and specificity to classify macadamia kernels

436 based on quality defects using PCA-LDA and NIRS.

Classes		1	2	3	4	5
Pre-Processing						
Raw	AC(%)	88.9	84.4	75.6	82.2	75.6
	SENS(%)	88.9	66.7	44.4	44.4	22.2
	SPEC(%)	88.9	88.9	83.3	91.7	88.9
SNV	AC(%)	80.0	68.9	88.9	75.6	75.6
	SENS(%)	66.7	55.6	55.6	11.1	33.3
	SPEC(%)	83.3	72.2	97.2	91.7	86.1
2nd Derivative	AC(%)	82.2	73.3	86.7	88.9	75.6
	SENS(%)	66.7	44.4	77.8	66.7	11.1
	SPEC(%)	86.1	80.6	88.9	94.4	91.7

437 SNV= standard normal variate; AC= accuracy; SENS= sensitivity; SPEC= specificity.

438 1=good, marketable kernels without defects; 2=kernels with discoloration; 3=immature

439 kernels; 4=kernels affected by mold; and 5=kernels with insect damage.

440

441

442

443 **Table 2.** Values of accuracy, sensitivity and specificity to classify macadamia kernels

444 based on quality defects using GA-LDA and NIRS.

Classes		1	2	3	4	5
Pre-Processing						
Raw	AC(%)	86.7	82.2	86.7	86.7	82.2
	SENS(%)	66.7	66.7	55.6	66.7	55.6
	SPEC(%)	91.7	86.1	94.4	91.7	88.9
SNV	AC(%)	97.8	84.4	88.9	91.1	84.4
	SENS(%)	88.9	88.9	55.6	77.8	55.6
	SPEC(%)	100	83.3	97.2	94.4	91.7
2nd Derivative	AC(%)	91.1	75.6	84.4	86.7	68.9
	SENS(%)	66.7	44.4	44.4	55.6	55.6
	SPEC(%)	97.2	83.3	94.4	94.4	72.2

445 SNV= standard normal variate; AC= accuracy; SENS= sensitivity; SPEC= specificity.

446 1=good, marketable kernels without defects; 2=kernels with discoloration; 3=immature

447 kernels; 4=kernels affected by mold; and 5=kernels with insect damage.

448

449

450

451 **Table 3.** Selected variables for GA-LDA to classify macadamia kernels using different
 452 pre-processing.

Pre-processing	Selected variables (nm)
Raw	882; 886; 946; 990; 1171; 1395; 1429; 1511; 1622; 1664; 1942; 1979; 2075; 2187; 2260; 2328
SNV	866; 1020; 1173; 1280; 1485; 1578; 1789; 1975; 1987; 2083; 2170; 2277; 2300; 2388; 2451
2nd Derivative	894; 898; 1078; 1251; 1335; 1436; 1488; 1952; 1964; 2126; 2328; 2356

453 SNV=standard normal variate

454

455

456 **Table 4.** Values of accuracy to classify macadamia kernels based on quality parameters

457 using PCA-LDA, GA-LDA and TD-NMR spectroscopy.

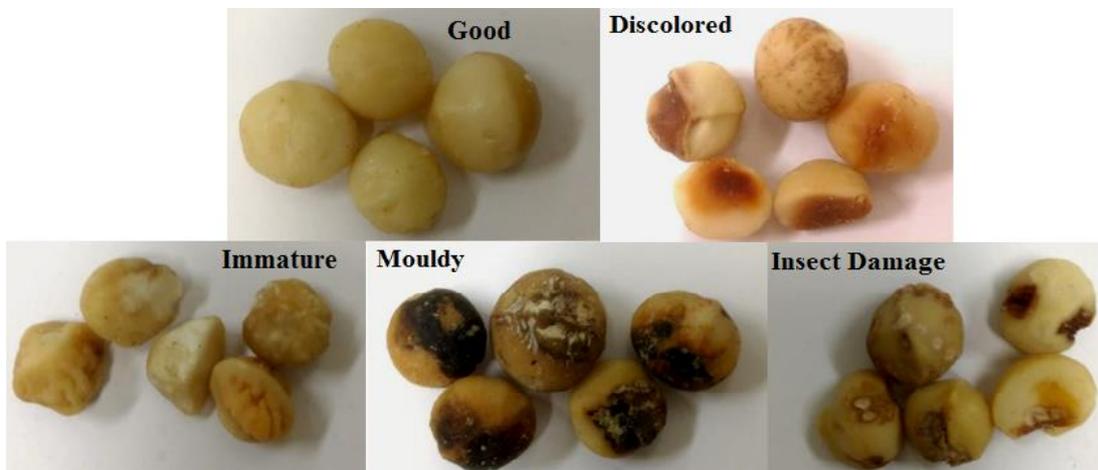
Classes	1	2	3	4	5	
Pre-Processing						
Nil	PCA-LDA					
	Training (%)	64.3	35.7	42.9	85.7	64.3
	Validation (%)	16.7	33.3	16.7	66.7	83.3
	GA-LDA					
	Training (%)	64.3	50.0	35.7	64.3	50.0
	Validation (%)	66.7	16.7	66.7	66.7	100

458 1=good, marketable kernels without defects; 2=kernels with discoloration; 3=immature

459 kernels; 4=kernels affected by mold; and 5=kernels with insect damage.

460

461

462 **Figures**

463

464 **Figure 1.** Macadamia kernels quality defects: 1=good, marketable kernels without

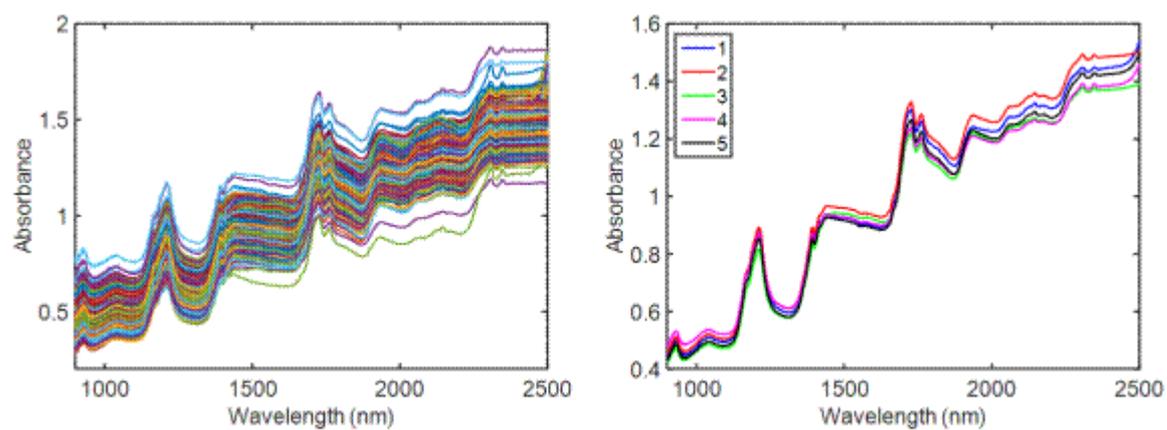
465 defects; 2=kernels with discoloration; 3=immature kernels; 4=kernels affected by mold;

466 and 5=kernels with insect damage.

467

468

469



470

471 **Figure 2.** Raw NIR spectra (a) and average NIR spectra (b) of macadamia kernels.

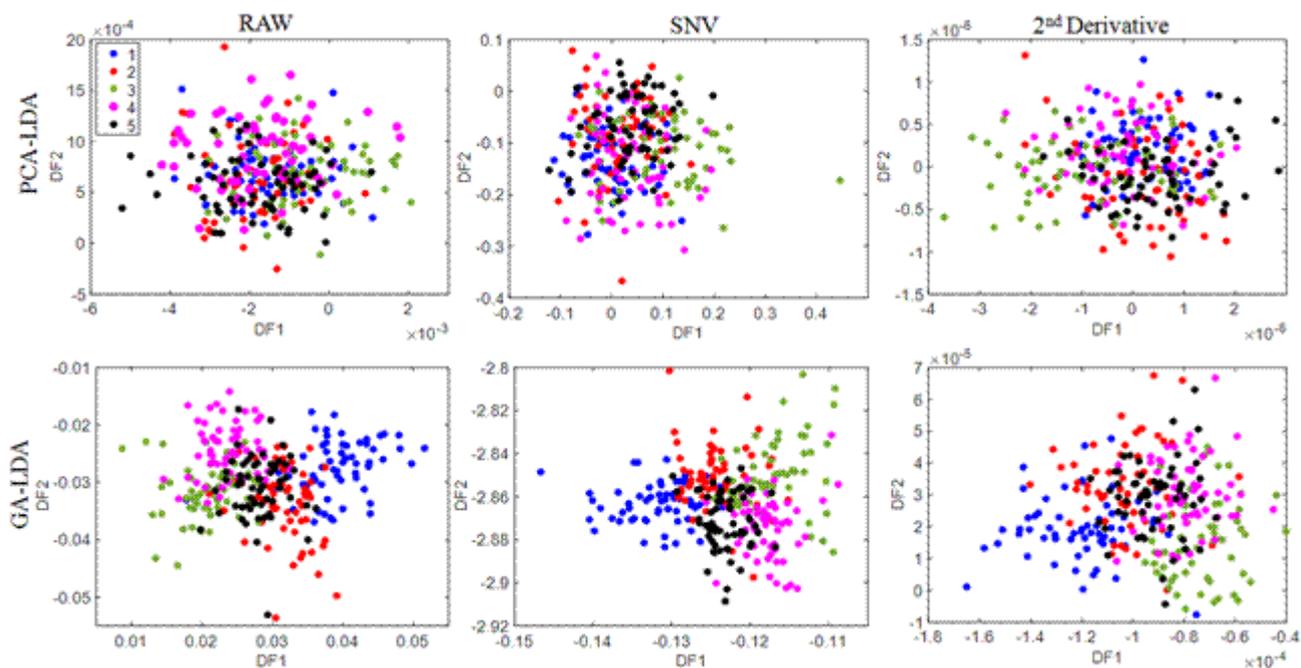
472 1=good, marketable kernels without defects; 2=kernels with discoloration; 3=immature

473 kernels; 4=kernels affected by mold; and 5=kernels with insect damage.

474

475

476

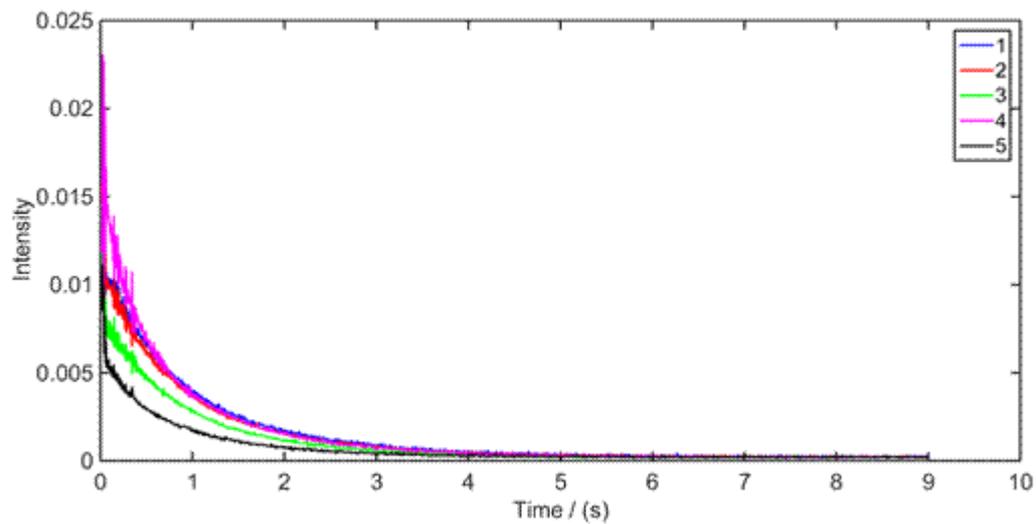


477

478 **Figure 3.** Discriminant function (DF) plot of PCA-LDA and GA-LDA with raw NIR
 479 spectra of macadamia kernels, SNV and 2nd derivative Savitzky-Golay. 1=good,
 480 marketable kernels without defects; 2=kernels with discoloration; 3=immature kernels;
 481 4=kernels affected by mold; and 5=kernels with insect damage.

482

483



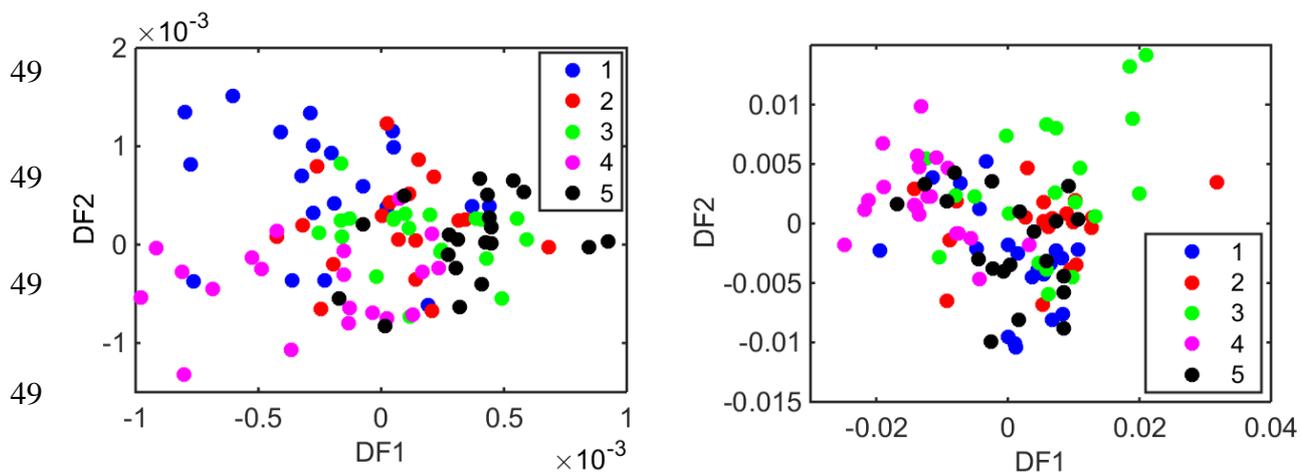
484

485 **Figure 4.** Typical CPMG decay curves of macadamia kernels with different quality
486 defects. 1=good, marketable kernels without defects; 2=kernels with discoloration;
487 3=immature kernels; 4=kernels affected by mold; and 5=kernels with insect damage.

488

489

490



495 **Figure 5.** Discriminant function (DF) of PCA-LDA (A) and GA-LDA (B) with raw
496 TD-NMR spectra of macadamia kernels. 1=good, marketable kernels without defects;
497 2=kernels with discoloration; 3=immature kernels; 4=kernels affected by mold; and
498 5=kernels with insect damage.

499

500