Article

Non-destructive assessment of the oxidative stability of intact macadamia nuts during the drying process by near-infrared spectroscopy

Carvalho, Livia C., Leite, Marcondes L., Medeiros-De-morais, Camilo De lelis, Lima, Kassio M.G. and Teixeira, Gustavo H.A.

It is advisable to refer to the publisher's version if you intend to cite from the work.
http://dx.doi.org/10.1016/j.lwt.2018.12.056

For more information about UCLan's research in this area go to http://www.uclan.ac.uk/researchgroups/ and search for <name of research Group>.

For information about Research generally at UCLan please go to http://www.uclan.ac.uk/research/

CLoK
Central Lancashire online Knowledge
www.clok.uclan.ac.uk

Non-destructive assessment of the oxidative stability of intact macadamia nuts during the drying process by near-infrared spectroscopy

Lívia C. Carvalho[1], Marcondes L. Leite[2,4], Camilo L. M. Morais[3], Kássio M. G. Lima[4], Gustavo H. A. Teixeira[5,*]

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

[2]Instituto Federal de Educação, Ciência e Tecnologia do Maranhão (IFMA), Campus São Luís-Maracanã. Avenida dos Curiós, s/n, Vila Esperança, CEP: 65.095-460, São Luís, Maranhão, Brazil.

[3]University of Central Lancashire, School of Pharmacy and Biomedical Sciences, Preston, Lancashire, PR1 2HE, United Kingdom.

[4]Universidade Federal do Rio Grande do Norte (UFRN), Instituto de Química, Química Biológica e Quimiometria, Avenida Senador Salgado Filho, n° 3000, Bairro de Lagoa Nova, CEP: 59.078-970, Natal, Rio Grande do Norte, Brazil.

[5]Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias (FCAV), Campus de Jaboticabal. Via de Acesso Prof. Paulo Donato Castellane s/n, CEP: 14.884-900, Jaboticabal, São Paulo, Brazil. Telephone: +55 16 3209-7532

*Corresponding author: gustavo@fcav.unesp.br

22

Abstract

We have developed a rapid non-destructive method to assess the oxidative stability of intact macadamia nuts using near-infrared spectroscopy (NIRS). Intact macadamia nuts of the cultivars HAES 344 'Kau', HAES 660 'Keaau', IAC 4-12 B, and IAC Campinas B were harvested and immediately oven-dried for 4 days at 30 ℃, 2 days at 40 ℃, and 1 day at 60 ℃ to achieve 1.5% kernel moisture content. At each drying step nuts were withdrawn and their moisture content, peroxide value (PV), and acidity index (AI) determined. The best partial least square model for PV prediction was obtained using the Savitzky-Golay (SG) second derivative resulting in a standard error of prediction (SEP) of 0.55 meq·kg^{-1} and a coefficient of determination (R^2_C) of 0.57. The best AI prediction-model result was obtained using the SG second derivative (SEP = 0.14%, R^2_C = 0.29). Based on the maximum quality limits of 3 meq·kg^{-1} for PV and 0.5% for AI, the SEP values represented 18% and 28%, respectively. Therefore, the prediction method can be considered useful since the errors are lower than the quality limits. Thus, NIRS can be used to assess the oxidative stability of intact macadamia kernels.

Keywords: peroxide value, acidity index, *Macadamia integrifolia* Maiden & Betche, principal component analysis.

1. Introduction

The delicious mild flavor and crispy texture of lightly roasted macadamia kernels have made them one of the most appreciated nuts in the world (Wall, 2013). High quality kernels contain 72–78% oil (Cavaletto, 1983), of which more than 77% corresponds to monounsaturated fatty acids,

predominantly monounsaturated fatty acids, while only around 16% are saturated fatty acids (Wood and Garg, 2010). This composition plays an important role in their quality, particularly in the organoleptic properties that make macadamia nuts so desirable (Silva, Maximo, Marsaioli Jr. & Silva, 2007).

Due to their high oil content, macadamia nuts are very susceptible to the occurrence of rancidification in the kernels, which causes objectionable flavors and odors in food products (Ramalho & Jorge, 2006; Silva et al., 2011). In addition, macadamia nuts contain the low levels of tocopherol antioxidants (~ 0.6–2.8 $\mu g \cdot g^{-1}$), (Wall, 2013).

Moisture content strongly influences early rancidity in macadamia nuts, and therefore, to retard rancidification and extend shelf-life, shelled nuts should be protected from moisture and oxygen during storage (Wall, 2013). Since the oxidative stability of the kernels is related to their moisture content, this parameter must be monitored to ensure drying to below 1.5% (Cavaletto, 1981; Silva et al., 2005; Borompichaichartkul et al., 2009). At this moisture level the water activity (aw) is equal or less than 0.3, which represents the optimum point for oxidative stability of dehydrated foods (Cavaletto, 1981; Wall, 2013). In addition to this, the maximum stability of macadamia nut corresponds to the minimum integral entropy zone (Dominguez et al., 2007), which corresponds to a range of aw from 0.36 to 0.44 (1.2-1.6% moisture). Therefore, 1.5% moisture content is fundamental to assure the oxidative stability of macadamia nuts.

Since freshly harvested nuts have a moisture content higher than 30% (Pankaew et al., 2016), drying should be started soon after harvesting to reduce the moisture content to prevent hydrolytic rancidity due to the nuts' high oil content (Mason and Wills, 2000) and/or mold development, such as *Colletotrichum gloeosporioides* and *Botrytis* sp. (Dierberger and Marino Netto, 1985).

The most useful analytical parameters to determine the degree of oxidation are peroxide value (PV) and acidity index (AI) (Wolff, 1997). PV is related to the formation of peroxides in unsaturated fats, due to the breaking of the double bonds, which generates short-chain volatile products

71   responsible for rancid odors. AI is related to the acidification of fats due to enzymatic reactions,

72   generating free fatty acids that could have unpleasant taste (Wolff, 1997). PV and AI can change at

73   high temperatures and can also depend on the cultivars. For example, PV changes on heating as it

74   decomposes into highly unstable secondary oxidation products while AI is influenced by heating due

75   to the reduced kernel-water content and/or altered enzymatic activity (Bai et al., 2017).

76   AI and PV must comply with the quality standards established for cold-pressed and non-

77   refined macadamia oils which require a maximum PV value of 3.5 meq·kg^{-1} and a maximum AI

78   value of 0.5% (SAMAC, 2015). The standard methods for PV and AI analyses are slow and time-

79   consuming, requiring precise quantification of the sample, dissolution in solvents, and titration with

80   standardized solutions, and are also relatively costly when used for industrial process monitoring

81   (Cozzolino, Murray, Chree & Scaife, 2005).

82   Near-infrared spectroscopy (NIRS) is widely employed for oxidation and moisture content

83   determination in oilseeds and grains and has the potential to become a powerful tool in lipid

84   oxidation analysis, especially with chemometric statistical evaluations. Non-destructive techniques

85   have great potential for shelled- and unshelled- macadamia nut sorting, and NIRS is such an

86   alternative, due to its long-term application to assess food-product quality parameters (Osborne,

87   Fearn & Hindle, 1993).

88   Canneddu, Júnior & Teixeira (2016) used FT-NIR spectroscopy without any pre-processing

89   to study the quality of shelled- and unshelled macadamia nuts, obtaining a coefficient of

90   determination (R^2_p) of 0.72 for PV prediction and an R^2_p of 0.80 for AI prediction. Guthrie, Greensill,

91   Bowden & Walsh (2005), evaluated the use of NIRS to assess the quality of macadamia kernels and

92   found an acceptable oil content prediction (root mean square error of cross validation $RMSE_{CV}$ =

93   2.5% and $R^2 > 0.98$), and moisture content prediction ($RMSE_{CV} < 0.2\%$, and $R^2 > 0.97$).

As NIRS is a fast and non-destructive method used in industrial quality control processes, the objective of this study was to evaluate its feasibility as an analytical method to improve the quality control of macadamia-nuts in post-harvest operations.

2. Materials and Methods

2.1. Plant Material

Twelve kilograms (kg) of intact dehusked macadamia (*Macadamia integrifolia* Maiden & Betche) nuts from each of the following cultivars, HAES 344 'Kau', HAES 660 'Keaau', IAC 4-12 B, and IAC Campinas B, were obtained from a commercial orchard located in Jaboticabal, São Paulo, Brazil, (21º 10' 14" South, 48º 37' 45" West, 600 m altitude) during the 2017 harvest season (March, April, and May).

2.2. Experimental set-up

Immediately after the harvests (n=3), 4 kg of intact dehusked nuts were submitted to oven-drying (Innova 4230, Edison, USA) in four steps: *i.* control (without drying), *ii.* 4 days at 30 °C, *iii.* 2 more days at 40 °C, and *iv.* 1 more day at 60 °C, to achieve the kernel moisture content of 1.5% stated by Mason (1995). The drying procedure was similar to what the macadamia industry uses in Brazil.

Initially (day 0) and at each drying step (4, 6, and 7 days), intact nuts (40 g) were withdrawn from the oven and the shell manually removed using a vice (N3, Schulz, Brazil). Unshelled nuts had their moisture content (%) determined following the A.O.A.C. (1997) standard method, Table 2.

Our experiment was set up as a completely randomized block design in a factorial arrangement 3 (harvests: March, April, and May) x 4 (cultivars: HAES 344 'Kau', HAES 660 'Keaau', IAC 4-12 B, and IAC Campinas B) $\times$ 3 (drying steps: 0 – control, 4 days at 30 °C, 2 more days at 40 °C, and 1 more day at 60 °C) with 5 repetitions, totaling 240 unshelled nut samples.

2.3. NIR spectra acquisition

The NIR spectra were taken on day 0, 4, 6, and 7 of the drying steps, on the surface of ~ 40 grams of unshelled macadamia nuts from each cultivar. The nuts were place into a measuring cup and two NIR spectra were obtained on the range 866 – 2,530 nm, at a resolution of 2 nm and 64 scans, after temperature stabilization at ~25 °C, using a Bruker NIR spectrometer (Model Tango, Ettlingen, Germany) in reflectance mode. The NIR spectra were averaged, and the mean spectra were processed, and the calibration models were performed using the MATLAB®, R2012b (MathWorks, USA) software.

2.4. Reference analyses: PV and AI

After drying, all samples were frozen at −20 °C, stored into plastic bags (~ 40 g of nuts per period and cultivar), to avoid oxidation. The oil present in the nuts were extracted daily to avoid supplementary oxidation, using the procedure reported by Canneddu, Júnior & Teixeira (2016). Briefly, the kernels were wrapped in nylon fabric and put into a stainless-steel vial with a tightly adjusted stainless-steel piston. The piston was pressed until free oil could be seen. The oil was rapidly transferred to a Falcon tube (BD Falcon™, BD Biosciences, Mass., USA) and used for the oxidation analysis.

PV: The peroxide value was determined using the official method published by the A.O.A.C. (1997). Briefly, 5 g of freshly pressed macadamia oil was poured into a 125 mL Erlenmeyer. Thirty mL of acetic acid-chloroform solution (3:2 v/v) was added and the flask was agitated until oil dissolution. Then, 0.5 mL of saturate potassium iodide solution was added, mixed, and let to rest for 1 minute. Thirty mL of distilled water was added, and the solution titrated with 0.01 N sodium thiosulfate. PV was expressed as active-oxygen milliequivalents per kilogram (meq·kg^{-1}). The descriptive statistics of all samples is shown in Table 1.

AI: The acidity index was also determined using the A.O.A.C. (1997) official method. Five mL of freshly pressed macadamia oil was poured into a 125 mL Erlenmeyer and 25 mL of ether-

143 ethanol (2:1 v/v) solution was added. NaOH 0.01 M was used to neutralize the free fatty acids

144 present in the oil with phenolphthalein (0.1%) as indicator. AI was expressed as the percentage of

145 oleic acid (%). The descriptive statistics of all samples are shown in Table 1.

146 **2.5. Data analysis**

147 *2.5.1. Chemometrics: multivariate analysis*

148 All the NIR spectra were handled, and the calibration models developed, using the

149 MATLAB®, R2012b (MathWorks, USA) software with the PLS Toolbox version 7.9.3 (Eigenvector

150 Research, Inc., USA). Prior to the spectral pre-processing, all NIR spectra were analyzed to identify

151 and eliminate outliers based on Hotelling's T^2 and Q-residuals statistics (Bro and Smilde, 2014).

152 NIR datasets are often very large and highly complex and, consequently, need to be pre-

153 processed. Therefore, prior to model development, the full spectra were pre-processed using

154 multiplicative scatter correction (MSC), standard normal variate (SNV), Savitzky-Golay smoothing

155 (SG), and derivatives. Then, the spectral datasets were correlated with the PV and AI values using

156 Partial Least Squares (PLS) regression and full cross-validation. The NIR spectra were divided into

157 two groups: the calibration set (n=154) and the validation set (n=66), using the classic Kernnard–

158 Stone selection algorithm (Kennard and Stone, 1969). In order to evaluate the performance of the

159 calibration models, the root mean square error of cross-validation ($RMSE_{CV}$) and root mean square

160 error of prediction ($RMSE_P$) were calculated, according to the following equation:

161
$$\text{RMSEC or RMSEP} = \sqrt{\frac{\sum_{i=1}^{np}(yi-yi')2}{n-K-1}}$$

162 Where: *yi* is the value predicted by the multivariate model, *yi'* the reference value, and

163 *n* the number of samples.

164 The performance of the calibration models was also evaluated based on the

165 determination coefficient R^2, both for the calibration and validation sets (Pasquini, 2003).

166 *2.5.2. Univariate analysis*

167   The data was subjected to analysis of variance (ANOVA) using the Agrostat (Barbosa and

168   Maldonado Júnior, 2015) software and the means were compared using Tukey's test with 5%

169   probability.

170

171   **3. Results and Discussion**

172   **3.1. Nut quality during drying**

173   The moisture, dry matter contents (%), PV and AI of the unshelled macadamia nuts of the

174   different cultivars, determined during drying, are shown in Table 2.

175   No significant moisture content differences were observed between nuts from different

176   cultivars, but during the drying process moisture content became significantly lower (Table 2). The

177   moisture content between cultivars ranged from 5.97% in HAES 344 to 7.24% in IAC Campinas B.

178   During the drying process the highest moisture content was observed on day 0 (13.81%); after 4 days

179   at 30 °C this value significantly ($P < 0.05$) decreased to 6.09%. The moisture content decreased to

180   4.13% after 2 days at 40 °C, and to 2.62% after 1 day at 60 °C, but without significant differences.

181   The dry matter content showed a similar trend, but in the opposite direction, increasing during drying

182   due to the dehydration process (Table 2).

183   According to Mason (2000) and Cavaletto (1983), macadamia nuts at harvest can have a

184   moisture content as high as 30% and it is essential to reduce it to 1.5% to prevent hydrolytic activity

185   and mold development. O'Hare, Hidden, Burton & Salmon (2004), reported that the nut-in-shell

186   moisture content should be reduced to 8–10% within two weeks of harvest to prevent quality

187   deterioration, mainly to control mold development, rancidity, off-flavors, and reduced shelf-life. In

188   this regard, only the nuts from day 0 presented a moisture content above this range, and after only 4

189   days at 30 °C the moisture content reached 6.09%, inside the 8–10% range.

190   The moisture content control during macadamia nut handling is extremely important as

191   rancidity can occur rapidly (Cavaletto, 1981; Cavaletto, 1986; Wall, 2013). According to Kaijser,

Dutta & Savage (2000), macadamia oil stability ranged from 3.6 to 19.8 h using the Rancimat test. Macadamia nuts are susceptible to rancidification due to their high oil content. Cavaletto (1983) reported an oil content of 72% and Kaijser, Dutta & Savage (2000) observed a range from 69 to 78%. In addition, the macadamia oil is highly monounsaturated (Ako, Okoda & Gray, 1995) and contains high levels of oleic (18:1), palmitoleic (16:1), and palmitic (16:0) acids (Kaijser, Dutta & Savage, 2000). Therefore, because drying was conducted immediately after harvest it resulted in a rapid moisture content reduction that improved the oxidative stability of the kernel, as evidenced by the low PV and AI values (Table 2).

No significant PV differences were observed neither from different cultivars nor during the drying process (Table 2). PV ranged from 1.08 meq·kg^{-1} in the nuts from the IAC 4-12 cultivar to 0.73 meq·kg^{-1} in those from HAES 344 (Table 2). During drying the PV ranged from 1.27 in day 0 to 0.66 meq·kg^{-1} after 4 days at 30 °C, 2 days at 40 °C, and 1 day at 60 °C (Table 2). The observed PVs were very low compared to those of the macadamia quality standards. According to Mason, Nottingham, Reid & Gathambiri (2004) the AMS Guidelines indicate a maximum PV of 5.0 meq·kg^{-1} for oil from raw and roasted macadamia kernels. According to SAMAC (2015), raw macadamia nuts must have PV less than 3.5 meq·kg^{-1}. Macadamia oil is considered moderately to completely rancid when its PV reaches 6.0 meq·kg^{-1} (McConachie, 1996).

PV can be influenced by the cultivar as each cultivar has a different nut polyunsaturated fatty acid composition (Kaijser, Dutta & Savage, 2000). Processing also influences PV. Canneddu, Júnior & Teixeira (2016), reported lower PVs (5.6–5.5 meq·kg^{-1}) in freshly harvested intact and ground macadamia nuts, when compared to half kernels from late harvest (10.7–18.8 meq·kg^{-1}). Oxidative rancidity is very important in lipid stability and since macadamia nuts have a low concentration of polyunsaturated fatty acids (3.0–4.7%) peroxides production is generally low in unstored kernels (Kaijser, Dutta & Savage, 2000). However, it can be a quality problem during drying

216 (Borompichaichartkul, Luengsode, Chinprahast & Devahastin, 2009) and long-term storage

217 (Sinanoglou et al., 2014).

218   Regarding AI, no significant ($P > 0.05$) differences between different cultivars were observed

219 and during drying process the AI decreases significantly (Table 2) ranging from 0.75% in nuts from

220 the IAC Campinas B cultivar to 0.66% in those from HAES 344. During the drying process the

221 highest AI was observed at day 0 (1.0 %); after 4 days at 30 °C this value significantly ($P < 0.05$)

222 decreased to 0.63%. AI decreased to 0.61% after 2 days at 40 °C and to 0.56% after 1 day at 60 °C,

223 but without significant differences (Table 2). This reduction of AI during the drying process could be

224 related to the inactivation of the enzymes involved in the hydrolytic reactions (Silva et al., 2011),

225 which are directly related to the moisture content present in the kernel (Silva, Maximo, Marsaioli

226 Júnior & Silva, 2007). Although the drying process was conducted immediately after harvesting, AI

227 values were above the maximum levels recommended for macadamia oil.

228   According to SAMAC (2015), the maximum AI should be 0.5%. McConachie (1996)

229 considered that high quality macadamia oil should have AI of only 0.1–0.3%, but Arnett (1995)

230 considered an AI of 0.9% as acceptable. Therefore, hydrolytic rancidity seems to be a more

231 important factor for macadamia nuts quality, as its development of rancid flavors and odors can take

232 place rapidly even during prompt drying.

233

234 **3.2. NIR spectra description**

235   A preliminary analysis of the raw NIR spectra showed very similar trends for the macadamia

236 nuts from all cultivars. Carvalho et al. (2018) also observed similarities of different macadamia nut

237 NIR spectra and the classification of shelled nuts was only possible using sophisticate chemometric

238 techniques. Because the raw NIR spectra presented broad light scattering they were pre-processed

239 with MSC and SNV plus Savitsky-Golay smoothing to allow the identification of four main peaks at

240 1200, 1500, 1800, and 2000 nm. The absorption bands around 1200 nm arise from the second

241   overtones of CH stretching vibrations, while those at 1700–1800 nm are attributable to the first

242   overtones of CH stretching vibrations of –CH_3, –CH_2– and –HC=CH (Armenta and La Guardia,

243   2007). The wavelength region situated at 2200–2500 nm is mainly related to the oxidation and

244   hydrolytic degradation of lipids (Cozzolino, Murray, Chree & Scaife, 2005).

245   During the drying process the NIR spectra changed mainly in the regions where the water

246   bands are present (1400–1440 nm and 1900–1950 nm) (Sundaram, Kandala, Holser, Butts &

247   Windham, 2010), indicating that there was a reduction in moisture content, as can be seen in Table 2.

248   Principal component analysis (PCA) was used to observe the spectral differences between

249   drying processes and macadamia nut cultivars, helping to observe possible groupings between the

250   samples due to their similarities (Cozzolino, Cynkar, Shah & Smith, 2011). The scores of the two

251   main principal components (PCs) obtained with the NIR spectra without preprocessing can be

252   observed in Figure 1. Regarding the drying process, PC2 grouped the macadamia nut NIR spectra

253   from day 0 and 7 (Figure 1A), as there was a significant moisture content difference ($P < 0.05$)

254   between day 0 and day 7 (Table 2). It was not possible to observe any grouping among macadamia

255   nut cultivars (Figure 1B), with PC1 and PC2 representing 97% of the data explained variance. This

256   result indicates that there was similarity between macadamia nut cultivars, which may be related to

257   climatic factors and/or for sharing the same pollination since they originated from the same orchard

258   (Carvalho et al. 2018).

259

260   **3.3. Oxidative stability: PV and AI**

261   The PV of the samples ranged from 0.14 to 5.41 meq·kg^{-1} and the AI from 0.18 to 2.26%

262   (Table 1) in the calibration set. As shown in Table 3, the best PLS model for PV ($RMSE_C = 0.56$

263   meq.kg^{-1}, $R_c^2 = 0.57$, SEP = 0.55 meq.kg^{-1}, $RMSE_P = 0.60$ meq.kg^{-1}, $R^2_P = 0.002$) was obtained

264   when the NIR spectra were pre-processed with SG second derivative. The predicted vs. measured PV

265   values can also be seen in Figure 2. Canneddu, Júnior & Teixeira (2016) studying the quality of

266 unshelled macadamia nuts by NIRS found higher SEP values (3.45 meq·kg^{-1}), but with a greater

267 variation in the PV values (4.6–26.8 meq·kg^{-1}) of the calibration set. Better PV prediction by NIRS

268 was reported by Armenta and La Guardia (2007) in edible oils of different types and origins, with

269 RMSE$_p$ values of 1.87 meq·kg^{-1} and R^2_c of 0.99. However, intact macadamia nuts are a more

270 heterogeneous matrix as compared to vegetable oils and this could have affected the results,

271 especially the lower determination coefficients (Table 3).

272 In contrast, considering the maximum PV quality limit of 3.0 meq·kg^{-1} (SAMAC, 2015), the

273 SEP value (0.55 meq·kg^{-1}) represented 18% of this recommendation. Therefore, the PV prediction

274 method can be considered excellent as its total error was of magnitude 25% or less (McFarren,

275 Lishka & Parker, 1970).

276 Regarding AI, the best PLS model was obtained when the NIR spectra were pre-processed

277 using SG second derivative (RMSE$_c$ = 0.25%; R$_c^2$ = 0.56; SEP=0.29%; RMSE$_p$= 0.30%; R$_p^2$=0.018

278 with 7 latent variables), Table 3 and Figure 3. As indicated in Table 3, the SEP and RMSE$_P$

279 differences were not significant which could be taken as an indication that the prediction precision of

280 the PLS regression was high (Amodio, Ceglia, Chaudhrya, Piazzolla & Colellia, 2017), although R^2

281 was low. Similar RMSE$_P$ values (0.35%) were reported by Mba, Adewale, Dumont & Ngadi (2014)

282 in palm and canola oils with NIR spectra pre-processed using SG first derivative, but their R^2 was

283 much greater (0.99). Better results were reported by Armenta and La Guardia (2007) in olive oil

284 (RMSEP = 0.083% and R2 = 0.99); once again, the difference between food matrixes must be taken

285 into consideration. Although the R^2 values were below 0.70, considering the AI level of 0.5% as the

286 maximum quality limit of macadamia nuts (SAMAC, 2015), the SEP value represented 28% of this

287 limit. Consequently, the AI prediction as an analytical method can be considered acceptable as its

288 total error was of the magnitude of 50 % or less (McFarren, Lishka & Parker, 1970).

289 The moisture prediction results (Table 3, Figure 4) indicate that the best calibration models

290 for moisture content of unshelled nuts were obtained using SG + 2nd derivative (Rc^2 = 0.66; RMSEc

291 = 2.33%). Better results were reported by Guthrie, Greensill, Bowden & Walsh (2005) who found

292 moisture content prediction models with RMSEcv of 0.11% and Rc² of 0.79. Their results were

293 improved by using ground nuts (RMSEcv = 0.06% Rc² = 0.95), and these differences could be due to

294 the lack of moisture homogeneity within our intact kernels; grinding should have reduced this

295 variation. Sumdaram, Kandala, Holser, Butts & Windham. (2010) also reported better results for in

296 shell peanuts using NIRS (RMSEp = 1.38% and R²c = 0.98), but the moisture content range was

297 much higher (6.18 - 21.69 %) and should be taken into consideration.

298

299 **4. Conclusion**

300 Cultivars had little effect on the oxidative stability of intact unshelled macadamia nuts based

301 on PV and AI. On the other hand, the drying process affected mostly the AI as the PV did not present

302 any modifications during drying.

303 The NIR spectra were similar between cultivars, but the absorption bands changed during the

304 drying process and the characteristic oil bands could be seen when the MSC, SNV, and SG

305 smoothing derivatives were applied.

306 The PLS calibration models for PV and AI were considered useful since the error is lower of

307 the quality limits. Therefore, NIRS can be used to assess the oxidative stability of intact macadamia

308 nuts, but further investigation must be done to address other sources of variability to improve the

309 robustness of the prediction models.

310

314

315 **5. References**

316

317   Akinoso, R., Aboaba, S.A., & Olayanju TMA. (2010). Effects of moisture content and heat treatment

318   on peroxide value and oxidative stability of un-refined sesame oil. *African Journal of Food,*

319   *Agriculture, Nutrition and Development*, *10*, 4268-4285. Doi: 10.4314/ajfand.v10i10.62908

320   Ako, H., Okoda, D., & Gray D (1995). Healthful new oil from macadamia nuts. *Nutrition*, *11*, 286-

321   288.

322   Amodio, M.L., Ceglia, F., Chaudhrya, M.M.A., Piazzolla, F., & Colellia G. (2017). Potential of NIR

323   spectroscopy for predicting internal quality and discriminating among strawberry fruits from

324   different production systems. *Postharvest Biology and Technology*, *125*, 112–121. Doi:

325   10.1016/j.postharvbio.2016.11.013.

326   AOAC. (1997). *Official methods of analysis of the association of official analytical chemists*. (16th

327   ed.). Arlington: Patricia Cuniff.

328   Armenta, S., Garrigues, S., De la Guardia, M. (2007). Determination of edible oil parameters by near

329   infrared spectrometry. *Analytica Chimica Acta, 596*, 330-337. Doi: 10.1016/j.aca.2007.06.028

330   Arnett G. (1995). Macadamia oil. *Australian Macadamia Society News Bulletin*, *22*: 23.

331   Bai, S.H., Darby, I., Nevenimo, T., Hannet, G., Hannet, D., Poienou, M., Grant, E., Brooks, P.,

332   Walton, D., Bruce, R., & Wallace HM. (2017). Effects of roasting on kernel peroxide value, free

333   fatty acid, fatty acid composition and crude protein content. *Plos One, 12*, 1-14. Doi:

334   10.1371/journal.pone.0184279

335   Barbosa, J.C., Maldonado Júnior, W. (2015). *Experimentação agronômica & Agroestat - Sistema*

336   *para análises estatísticas de ensaios agronômicos*. Jaboticabal: Gráfica Multipress LTDA. 396 p

337   Bro, R., & Smilde, A.K. (2014). Principal component analysis. *Analitical Methods, 6*, 2812-2831. Doi:

338   10.1039/C3AY41907J

339 Borompichaichartkul, C., Luengsode, K., Chinprahast, N., & Devahastin, S. (2009) Improving

340 quality of macadamia nut (*Macadamia integrifolia*) through the use of hybrid drying process.

341 *Journal of Food Engineering*, *93*, 348-353. Doi: 10.1016/j.jfoodeng.2009.01.035.

342 *Canneddu, G., Júnior, L.C., & Teixeira, G.H.A. (2016). Quality evaluation of shelled and unshelled

343 macadamia nuts by means of near-infrared spectroscopy (NIR). *Journal of Food Science, 81*, 1613-

344 1621. Doi: 10.1111/1750-3841.13343.

345 Carvalho, L.C., Morais, C., Lima, K., Leite, G.W.P., Oliveira, G.S., Casagrande, I.P., Santos Neto,

346 J.P., & Teixeira, G.H.A. (2018). Using intact nuts and near infrared spectroscopy to classify

347 macadamia cultivars. *Food Analytical Methods,* 11, 1857-1866. Doi: 10.1007/s12161-017-1078-9

348 Cavaletto CG (1983). Macadamia nuts. In H.T.Jr. Chang (Ed.), *Handbook of tropical foods*, p.361-

349 397. New York: Marcel Dekker Inc.

350 *Cavaletto, C.G. (1981). Quality evaluation of macadamia nuts. In G. Charalambous., & G. Inglett

351 (Eds.), *Quality of foods and beverages: chemistry and technology*, vol. 2 (pp. 71–82). New York:

352 Academic Press.

353 Cozzolino, D., Murray, I., Chree, A., & Scaife, J.R. (2005). Multivariate determination of free fatty

354 acids and moisture in fish oils by partial least-squares regression and near-infrared spectroscopy.

355 *LWT*, *38*, 821-828. Doi: 10.1016/j.lwt.2004.10.007.

356 Cozzolino, D.; Cynkar, W.; Shah, N.; & Smith, P.A. (2011) Can spectroscopy geographically

357 classify Sauvignon blanc wines from Australia and New Zealand? *Food Chemistry, 126*, 673-678.

358 *Guthrie. J., Greensill, C., Bowden, R., & Walsh, K. (2004). Assessement of quality defects in

359 macadamia kernels using NIR spectroscopy. *Australian Journal of agricultural Research*, *55*, 471-

360 476. Doi: 10.1071/AR03179. Doi: 0.1016/j.foodchem.2010.11.005.

361 Domínguez, I., Azuara, E., Vernon-Carter, E.J., Beristain, C.I. (2007). Thermodynamic analysis of

362 the effect of water activity on the stability of macadamia nut. *Journal of Food Engineering*, *81*, 566-

363 571. Doi:10.1016/j.jfoodeng.2006.12.012

364 *Kaijser, A., Dutta, P., & Savage, G. (2000). Oxidative stability and lipid composition of macadamia

365 nuts grown in New Zealand. *Food Chemistry*, *71*, 67-70. Doi: 10.1016/S0308-8146(00)00132-1.

366 Kennard RW, & Stone LA (1969). Computer aided design of experiments. *Technometrics*, *11*, 137–

367 148.

368 Mason, R.L. (2000). Macadamia nut quality research: the processing challenge. *Food Australia*, *52*,

369 416-419.

370 Mason, R.L., & Wills, R.B.H. (2000). Macadamia nut quality research: the processing challenge.

371 *Food Australia*, *52*, 416-419.

372 Mason, R.L., Nottingham, S.M, Reid, C.E, & Gathambiri, C. (2004). The quality of macadamia

373 kernels stored in simulated bulk retail dispensers. *Food Australia*, *56*, 133-139.

374 Mba, O., Adewale, P., Dumont, M.J., & Ngadi, M. (2014). Application of near-infrared spectroscopy

375 to characterize binaryblends of palm and canola oils. *Industrial Crops and Products, 61*, 472–478.

376 Doi: 10.1016/j.indcrop.2014.07.037.

377 McConachie, I. (1996) Fats and oils as applied to macadamia nuts: a very basic chemistry lesson.

378 *Australian Macadamia Society News Bulletin*, *23*, 34.

379 McFarren, E.F., Lishka, R.J., & Parker, J.H. (1970). Criterion for judging acceptability of analytical

380 methods. *Analytical Chemistry*, *42*, 358-365. Doi: 10.1021/ac60285a009.

381 *O'Hare P, Hidden DV, Burton D, Salmon T. (2004). *Australian macadamia industry code of sound

382 *orchard practices*. Australian Macadamia Society, Lismore.

383 Pankaew, P., Janjai, S., Nilnont, W., Phusampao, C., Bala, B.K. (2016). Moisture desorption

384 isotherm, diffusivity and finite element simulation of drying of macadamia nut (*Macadamia

385 *integrifolia*). *Food and Bioproducts Processing*, *100*, 16–24. doi.org/10.1016/j.fbp.2016.06.007

386 Pasquini, C. (2003). Near infrared spectroscopy: fundamentals, practical aspects and analytical

387 applications. *Journal of Brazilian Chemistry Society, 14*, 198-219. Doi: 10.1590/s0103-

388 50532003000200006.

389 Ramalho, V.C., Jorge, N. (2006). Antioxidantes utilizados em óleos, gorduras e alimentos
390 gordurosos. *Química Nova*, *29*, 755-760. Doi: 10.1590/S0100-40422006000400023

391 SAMAC – Southern African Macadamia Growers' Association. (2015). *Raw macadamia products*
392 *quality specifications*. http://www.samac.org.za/index.php/standards [Acessed January 2018].

393 Silva, F.A., Marsaioli, A.J., Maximo, G.J., Silva, M.A.A.P., Goncalves, L.A.G. (2005). Microwave
394 assisted drying of macadamia nuts. *Journal of Food Engineering*, *77*, 550–558.

395 Silva, F.A., Maximo, G.J., Marsoioli, Junior, A., & Silva, M.A.A.P. (2007) Impacto da secagem com
396 microondas sobre o perfil sensorial de amêndoas de noz macadâmia. *Ciência e Tecnologia de*
397 *Alimentos*, *27*, 553-561.

398 Silva, F.A., Gonçalves, L.A.G., Damiani, C., Gonçalves, M.A.B., Soares Júnior, M.S., Marsaioli,
399 Júnior. A. (2011). Estabilidade oxidativa de amêndoas de noz macadâmia secas por micro-ondas com
400 ar quente. *Pesquisa Agropecuaria Tropical, 41*, 286-292. Doi: 10.1590/S0101-20612007000300020.

401 Sinanoglou, V.J., Kakkotou, K., Fotakis, C., Strati, I., Proestos, C., & Zoumpoulakis, P. (2014)
402 Monitoring the quality of γ-irradiated macadamia nuts based on lipid profile analysis and
403 Chemometrics. Traceability models of irradiated sample. *Food Research International*, *60*, 38-47.
404 Doi: 10.1016/j.foodres.2014.01.015.

405 Sundaram, J., Kandala, V.C., Holser, R.A., Butts, C.L., Windham, W.R. (2010). Determination of in-
406 shell peanut oil and fatty acid composition using near-infrared Reflectance spectroscopy. *Journal of*
407 *the American Oil Chemists' Society*, *87*, 1103–1114. Doi: 10.1007/s11746-010-1589-7.

408 Osborne BG, Fearn T, Hindle PH (1993). *Practical NIR spectroscopy with applications in food and*
409 *beverage analysis* (2nd ed.). Essex: Longman Group Burnt Mill, Harlow.

410 Wallace, H.M., & Walton, D.A. (2011). Macadamia (*Macadamia integrifolia*, *Macadamia*
411 *tetraphylla* and hybrids). In E.M. Yahia (Ed.), *Postharvest biology and technology of tropical and*
412 *subtropical fruits*, vol. 3 (pp.450-473). Cambridge: Woodhead Publishing Series in Food Sciences,
413 Technology and Nutrition: number 208

414   Wall, M.M (2013). Improving the quality and safety of macadamia nuts. In Harris, L.J. (Ed.),

415   *Improving the quality and safety of nuts* Cambridge: Woodhead Publishing Series in Food Science

416   Technology and Nutrition. 250: 274-296.

417   Wolff, J. P. (1997). Analysis and Determination of Lipids, In: *Analysis of Food Constituents* (pp.

418   175-220). Wiley – VCH Inc., Canada.

419   Wood, L.G., & Garg, M.L. (2011). Macadamia nuts (*Macadamia integrifolia* and *tetraphylla*) and

420   their use in hypercholesterolemic subjects (pp.717-725). In: Preedy, V.R., Watson, R.R., Patel, V.B.

421   (ed.). *Nuts & seeds in health and disease prevention*. Academic Press, London, UK.

422

423   *These references were fundamental for the discussion of the article, since they deal with

424   works similar to the above, using macadamia nuts, near infrared spectroscopy combined with

425   chemometrics and oxidative evaluation of nuts, clearly exposing the quality parameters of

426   macadamia nuts.

427

428 **Tables**

429

430 **Table 1.** Descriptive statistics of unshelled macadamia nuts of the calibration and validation sets

431 obtained using the classic Kernnard-Stone selection algorithm.

Description	Total	Mean	Maximum	Minimum	SD[a]
Calibration set	154*				
Peroxide value (meq.kg^{-1})	154	0.90	5.41	0.12	0.82
Acidity index (%)	154	0.68	2.26	0.20	0.35
Validation set	66*				
Peroxide value (meq.kg^{-1})	66	0.86	2.49	0.14	0.58
Acidity index (%)	66	0.60	1.65	0.18	0.30

432 [a]SD = standard deviation. *from the total of 240 samples, 20 were lost during oil extraction which

433 corresponds a total of 220 samples.

434

435

436

437

Table 2. Moisture, dry matter, peroxide value, and acidity index of unshelled macadamia
(*Macadamia integrifolia* Maiden & Betche) from different cultivars during drying process.

	Moisture (%)	Dry matter (%)	Peroxide value (meq.kg^{-1})	Acidity index (%)
Harvests (B)				
March	7.2±3.7	92.6±3.6	1.15±1.05	0.69±0.40
Aphril	3.7±5.3	94.4±3.7	0.84±0.54	0.66±0.28
May	2.6±1.5	97.4±1.5	0.66±0.35	0.61±0.32
Cultivars (C)				
IAC 4 12	7.1±7.9	92.8±7.9	1.08±0.67	0.72±0.37
IAC Campinas B	7.2±4.4	92.5±4.6	0.96±0.77	0.75±0.36
HAES 660	6.4±5.5	93.9±5.4	0.90±0.54	0.67±0.22
HAES 344	6.0±5.1	93.5±5.1	0.73±0.37	0.66±0.20
Drying period (D)				
0	13.8±6.9^a	86.2±6.4^c	1.27±0.97	1.01±0.43^a
4	6.1±2.7^b	93.3±3.1^b	0.93±0.61	0.63±0.27^b
6	4.1±1.5^b	95.9±1.5^{ab}	0.81±0.36	0.61±0.12^b
7	2.6±1.2^b	97.4±1.2^a	0.66±0.27	0.56±0.21^b
Interaction				
C x D	NS	NS	NS	NS

440 Average values with the same letter in the columns are not statistically different by Tukey's test

441 (p<0,05). Values in the column without letter are not statistically different by Tukey's test (P<0,05).

442 NS, non-significant interaction.

443

444

445

Table 3. Statistical parameters for the optimal prediction models for peroxide value, acidity index, and moisture of unshelled macadamia (*Macadamia integrifolia* Maiden & Betche) from different cultivars during drying process.

Peroxide value (PV)	mequiv. O_2 kg^{-1} units					
Pre- Processing	Calibration		Validation			
	$RMSE_c$	R_c^2	$RMSE_p$	SEP	Bias	RE(%)
Raw	0.78	0.18	0.47	0.42	0.21	66.57
SG+SNV	0.76	0.21	0.50	0.48	0.18	72.16
SG+MSC+baseline	0.78	0.17	0.51	0.45	0.23	72.33
SG+2nd derivative	0.56	0.57	0.60	0.55	0.26	86.04
Acidity Index (AI)	**% (w/w) units**					
Pre- Processing	Calibration		Validation			
	$RMSE_c$	R_c^2	$RMSE_p$	SEP	Bias	RE(%)
Raw	0.36	0.06	0.22	0.16	0.15	38.89
SG+SNV	0.36	0.08	0.23	0.16	0.16	40.93
SG+MSC+baseline	0.35	0.12	0.25	0.19	0.16	44.80
SG+2nd derivative	0.25	0.56	0.30	0.29	0.10	54.93
Moisture	**% (w/w) units**					
Pre- Processing	Calibration		Validation			
	$RMSE_c$	R_c^2	$RMSE_p$	SEP	Bias	RE(%)
Raw	3.21	0.23	3.01	3.03	0.12	49.65
SG+SNV	3.22	0.23	3.12	3.15	-0.01	51.51
SG+MSC+baseline	3.26	0.21	3.20	3.23	-0.15	52.83
SG+2nd derivative	2.13	0.66	3.23	3.26	-0.07	53.31
Dry Matter	**% (w/w) units**					
Pre- Processing	Calibration		Validation			
	$RMSE_c$	R_c^2	$RMSE_p$	SEP	Bias	RE(%)
Raw	3.30	0.26	3.07	3.09	-0.15	3.24
SG+SNV	3.30	0.25	3.17	3.20	0.01	3.34
SG+MSC+baseline	3.34	0.24	3.26	3.28	0.15	3.43
SG+2nd derivative	2.19	0.67	3.31	3.33	0.01	3.48

mequiv = milliequivalent; SG = Savitzky-Golay smoothing; SNV = standard normal variate; MSC = multiplicative scatter correction; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction; R_c^2 = coefficient of determination for calibration; SEP = standard error for prediction; RE = relative error.
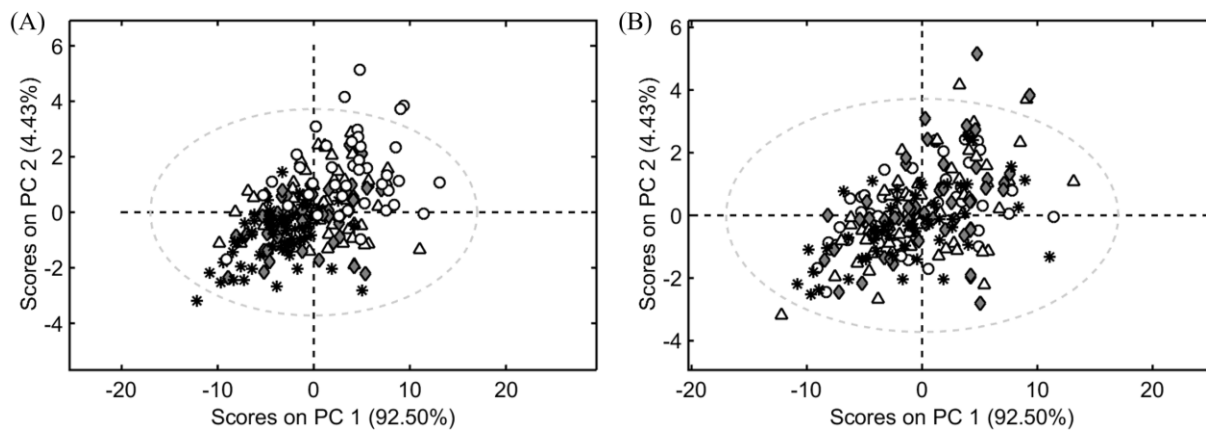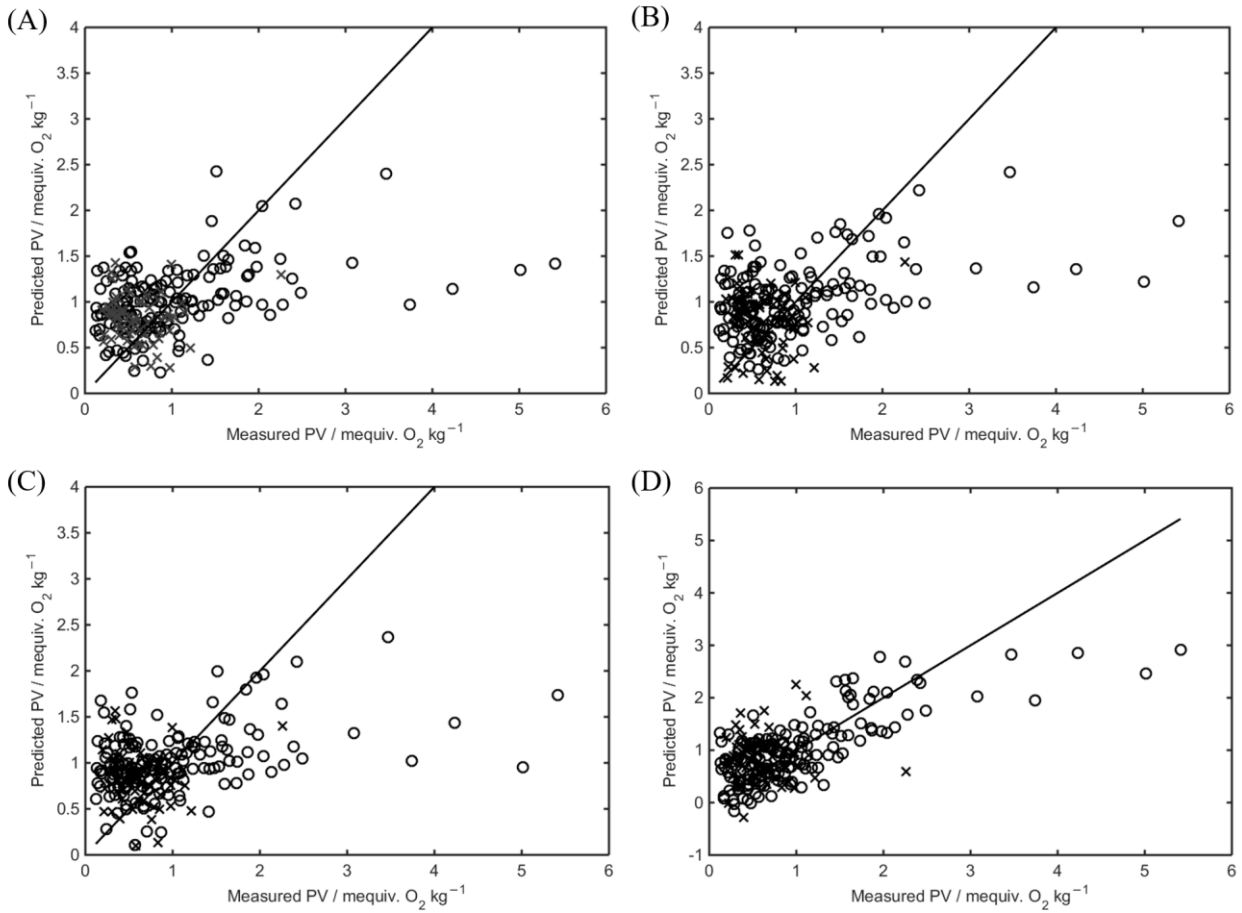
453

454

455 **Figures**

456

457

458

459

Figure 1. (A) scores of the PCA for the NIR spectra of drying process (Day 0 [○], 4 [▲], 6 [◊], 7

[*]); (B) scores of the PCA for the NIR spectra of unshelled macadamia (*Macadamia integrifolia*

Maiden & Betche) nuts from different cultivars (IAC 4-12 [◊], IAC Campinas B [*], HAES 660 [▲],
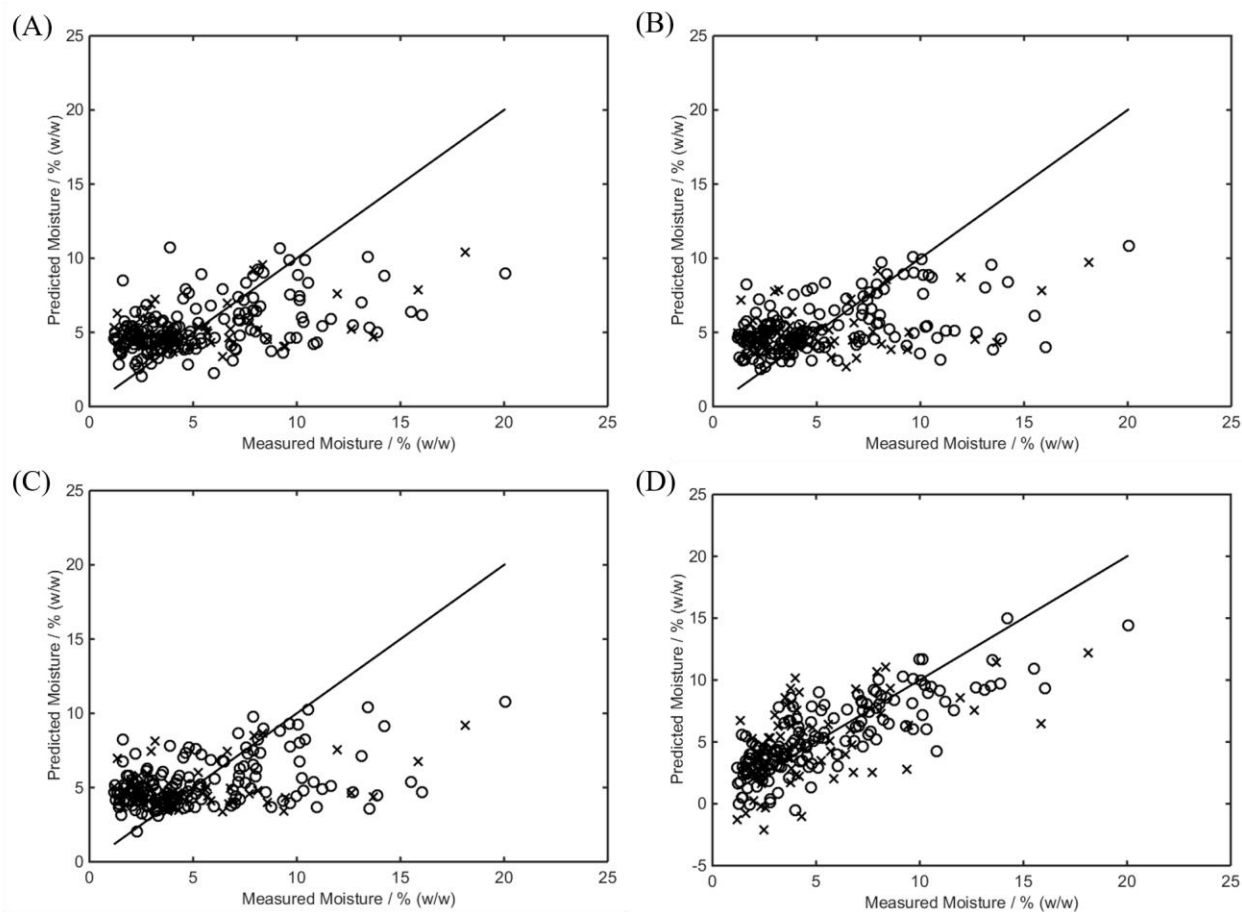
HAES 340 [○]).

464

Figure 2. Measured *versus* predicted peroxide value (PV) for the calibration (o) and validation (x)

sets using PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C)

SG+MSC+baseline; (D) SG+2nd derivative. SG = Savitzky-Golay smoothing; SNV = standard

normal variate; MSC = multiplicative scatter correction.

469
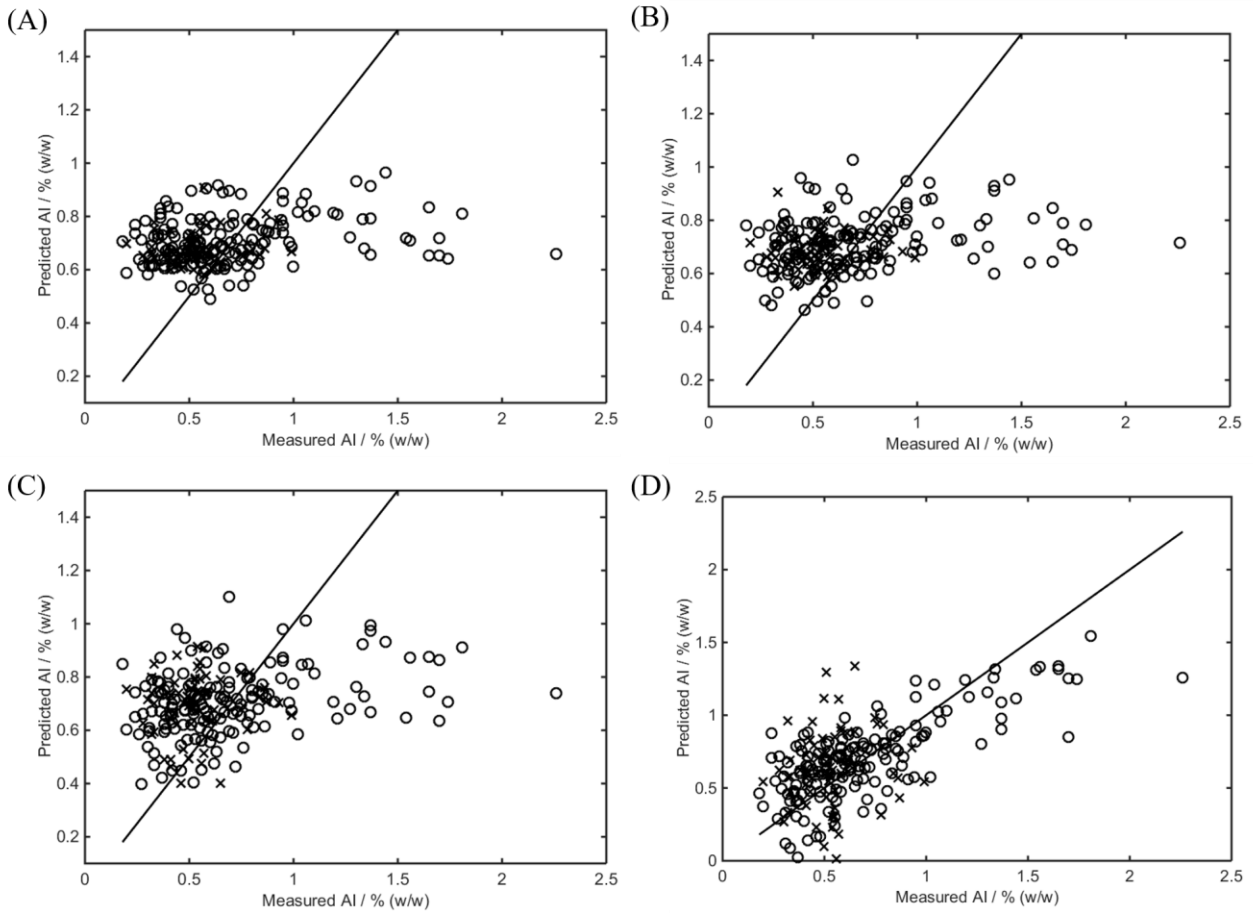
Figure 3. Measured *versus* predicted acidity index (AI) for the calibration (o) and validation (x) sets using PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C) SG+MSC+baseline; (D) SG+2nd derivative. SG = Savitzky-Golay smoothing; SNV = standard normal variate; MSC = multiplicative scatter correction

474

Figure 4. Measured *versus* predicted moisture for the calibration (o) and validation (x) sets using PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C) SG+MSC+baseline; (D) SG+2nd derivative. SG = Savitzky-Golay smoothing; SNV = standard normal variate; MSC = multiplicative scatter correction.