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

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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 °C, 2 days at 40 °C, and 1 day at 60 °C 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 At each drying step nuts were withdrawn and their moisture content, peroxide value (PV), and acidity index (AI) determined.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

responsible for rancid odors. AI is related to the acidification of fats due to enzymatic reactions, generating free fatty acids that could have unpleasant taste (Wolff, 1997). PV and AI can change at high temperatures and can also depend on the cultivars. For example, PV changes on heating as it decomposes into highly unstable secondary oxidation products while AI is influenced by heating due to the reduced kernel-water content and/or altered enzymatic activity (Bai et al., 2017).

AI and PV must comply with the quality standards established for cold-pressed and non-refined macadamia oils which require a maximum PV value of 3.5 $meq \cdot kg^{-1}$ and a maximum AI value of 0.5% (SAMAC, 2015). The standard methods for PV and AI analyses are slow and time-consuming, requiring precise quantification of the sample, dissolution in solvents, and titration with standardized solutions, and are also relatively costly when used for industrial process monitoring (Cozzolino, Murray, Chree & Scaife, 2005).

Near-infrared spectroscopy (NIRS) is widely employed for oxidation and moisture content determination in oilseeds and grains and has the potential to become a powerful tool in lipid oxidation analysis, especially with chemometric statistical evaluations. Non-destructive techniques have great potential for shelled- and unshelled- macadamia nut sorting, and NIRS is such an alternative, due to its long-term application to assess food-product quality parameters (Osborne, Fearn & Hindle, 1993).

Canneddu, Júnior & Teixeira (2016) used FT-NIR spectroscopy without any pre-processing to study the quality of shelled- and unshelled macadamia nuts, obtaining a coefficient of determination (R^2_p) of 0.72 for PV prediction and an R^2_p of 0.80 for AI prediction. Guthrie, Greensill, Bowden & Walsh (2005), evaluated the use of NIRS to assess the quality of macadamia kernels and found an acceptable oil content prediction (root mean square error of cross validation $RMSE_{CV}$ = 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) × 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.

118 **2.3. NIR spectra acquisition**

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

126 **2.4. Reference analyses: PV and AI**

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

134 *PV*: The peroxide value was determined using the official method published by the A.O.A.C.
135 (1997). Briefly, 5 g of freshly pressed macadamia oil was poured into a 125 mL Erlenmeyer. Thirty
136 mL of acetic acid-chloroform solution (3:2 v/v) was added and the flask was agitated until oil
137 dissolution. Then, 0.5 mL of saturate potassium iodide solution was added, mixed, and let to rest for
138 1 minute. Thirty mL of distilled water was added, and the solution titrated with 0.01 N sodium
139 thiosulfate. PV was expressed as active-oxygen milliequivalents per kilogram ($meq \cdot kg^{-1}$). The
140 descriptive statistics of all samples is shown in Table 1.

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

ethanol (2:1 v/v) solution was added. NaOH 0.01 M was used to neutralize the free fatty acids present in the oil with phenolphthalein (0.1%) as indicator. AI was expressed as the percentage of oleic acid (%). The descriptive statistics of all samples are shown in Table 1.

2.5. Data analysis

2.5.1. Chemometrics: multivariate analysis

All the NIR spectra were handled, and the calibration models developed, using the MATLAB®, R2012b (MathWorks, USA) software with the PLS Toolbox version 7.9.3 (Eigenvector Research, Inc., USA). Prior to the spectral pre-processing, all NIR spectra were analyzed to identify and eliminate outliers based on Hotelling's T^2 and Q-residuals statistics (Bro and Smilde, 2014).

NIR datasets are often very large and highly complex and, consequently, need to be pre-processed. Therefore, prior to model development, the full spectra were pre-processed using multiplicative scatter correction (MSC), standard normal variate (SNV), Savitzky-Golay smoothing (SG), and derivatives. Then, the spectral datasets were correlated with the PV and AI values using Partial Least Squares (PLS) regression and full cross-validation. The NIR spectra were divided into two groups: the calibration set (n=154) and the validation set (n=66), using the classic Kernnard–Stone selection algorithm (Kennard and Stone, 1969). In order to evaluate the performance of the calibration models, the root mean square error of cross-validation ($RMSE_{CV}$) and root mean square error of prediction ($RMSE_P$) were calculated, according to the following equation:

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

Where: *yi* is the value predicted by the multivariate model, *yi*' the reference value, and *n* the number of samples.

The performance of the calibration models was also evaluated based on the determination coefficient R^2, both for the calibration and validation sets (Pasquini, 2003).

2.5.2. Univariate analysis

The data was subjected to analysis of variance (ANOVA) using the Agrostat (Barbosa and Maldonado Júnior, 2015) software and the means were compared using Tukey's test with 5% probability.

3. Results and Discussion

3.1. Nut quality during drying

The moisture, dry matter contents (%), PV and AI of the unshelled macadamia nuts of the different cultivars, determined during drying, are shown in Table 2.

No significant moisture content differences were observed between nuts from different cultivars, but during the drying process moisture content became significantly lower (Table 2). The moisture content between cultivars ranged from 5.97% in HAES 344 to 7.24% in IAC Campinas B. During the drying process the highest moisture content was observed on day 0 (13.81%); after 4 days at 30 °C this value significantly ($P < 0.05$) decreased to 6.09%. The moisture content decreased to 4.13% after 2 days at 40 °C, and to 2.62% after 1 day at 60 °C, but without significant differences. The dry matter content showed a similar trend, but in the opposite direction, increasing during drying due to the dehydration process (Table 2).

According to Mason (2000) and Cavaletto (1983), macadamia nuts at harvest can have a moisture content as high as 30% and it is essential to reduce it to 1.5% to prevent hydrolytic activity and mold development. O'Hare, Hidden, Burton & Salmon (2004), reported that the nut-in-shell moisture content should be reduced to 8–10% within two weeks of harvest to prevent quality deterioration, mainly to control mold development, rancidity, off-flavors, and reduced shelf-life. In this regard, only the nuts from day 0 presented a moisture content above this range, and after only 4 days at 30 °C the moisture content reached 6.09%, inside the 8–10% range.

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

(Borompichaichartkul, Luengsode, Chinprahast & Devahastin, 2009) and long-term storage (Sinanoglou et al., 2014).

Regarding AI, no significant ($P > 0.05$) differences between different cultivars were observed and during drying process the AI decreases significantly (Table 2) ranging from 0.75% in nuts from the IAC Campinas B cultivar to 0.66% in those from HAES 344. During the drying process the highest AI was observed at day 0 (1.0 %); after 4 days at 30 °C this value significantly ($P < 0.05$) 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, but without significant differences (Table 2). This reduction of AI during the drying process could be related to the inactivation of the enzymes involved in the hydrolytic reactions (Silva et al., 2011), which are directly related to the moisture content present in the kernel (Silva, Maximo, Marsaioli Júnior & Silva, 2007). Although the drying process was conducted immediately after harvesting, AI values were above the maximum levels recommended for macadamia oil.

According to SAMAC (2015), the maximum AI should be 0.5%. McConachie (1996) considered that high quality macadamia oil should have AI of only 0.1–0.3%, but Arnett (1995) considered an AI of 0.9% as acceptable. Therefore, hydrolytic rancidity seems to be a more important factor for macadamia nuts quality, as its development of rancid flavors and odors can take place rapidly even during prompt drying.

3.2. NIR spectra description

A preliminary analysis of the raw NIR spectra showed very similar trends for the macadamia nuts from all cultivars. Carvalho et al. (2018) also observed similarities of different macadamia nut NIR spectra and the classification of shelled nuts was only possible using sophisticate chemometric techniques. Because the raw NIR spectra presented broad light scattering they were pre-processed with MSC and SNV plus Savitsky-Golay smoothing to allow the identification of four main peaks at 1200, 1500, 1800, and 2000 nm. The absorption bands around 1200 nm arise from the second

overtones of CH stretching vibrations, while those at 1700–1800 nm are attributable to the first overtones of CH stretching vibrations of $–CH_3$, $–CH_2–$ and –HC=CH (Armenta and La Guardia, 2007). The wavelength region situated at 2200–2500 nm is mainly related to the oxidation and hydrolytic degradation of lipids (Cozzolino, Murray, Chree & Scaife, 2005).

During the drying process the NIR spectra changed mainly in the regions where the water bands are present (1400–1440 nm and 1900–1950 nm) (Sundaram, Kandala, Holser, Butts & Windham, 2010), indicating that there was a reduction in moisture content, as can be seen in Table 2.

Principal component analysis (PCA) was used to observe the spectral differences between drying processes and macadamia nut cultivars, helping to observe possible groupings between the samples due to their similarities (Cozzolino, Cynkar, Shah & Smith, 2011). The scores of the two main principal components (PCs) obtained with the NIR spectra without preprocessing can be observed in Figure 1. Regarding the drying process, PC2 grouped the macadamia nut NIR spectra from day 0 and 7 (Figure 1A), as there was a significant moisture content difference ($P < 0.05$) between day 0 and day 7 (Table 2). It was not possible to observe any grouping among macadamia nut cultivars (Figure 1B), with PC1 and PC2 representing 97% of the data explained variance. This result indicates that there was similarity between macadamia nut cultivars, which may be related to climatic factors and/or for sharing the same pollination since they originated from the same orchard (Carvalho et al. 2018).

3.3. Oxidative stability: PV and AI

The PV of the samples ranged from 0.14 to 5.41 $meq \cdot kg^{-1}$ and the AI from 0.18 to 2.26% (Table 1) in the calibration set. As shown in Table 3, the best PLS model for PV ($RMSE_C$ = 0.56 $meq.kg^{-1}$, 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 when the NIR spectra were pre-processed with SG second derivative. The predicted vs. measured PV values can also be seen in Figure 2. Canneddu, Júnior & Teixeira (2016) studying the quality of

unshelled macadamia nuts by NIRS found higher SEP values (3.45 meq·kg^{-1}), but with a greater variation in the PV values (4.6–26.8 meq·kg^{-1}) of the calibration set. Better PV prediction by NIRS was reported by Armenta and La Guardia (2007) in edible oils of different types and origins, with $RMSE_p$ values of 1.87 meq·kg^{-1} and R^2_c of 0.99. However, intact macadamia nuts are a more heterogeneous matrix as compared to vegetable oils and this could have affected the results, especially the lower determination coefficients (Table 3).

In contrast, considering the maximum PV quality limit of 3.0 meq·kg^{-1} (SAMAC, 2015), the SEP value (0.55 meq·kg^{-1}) represented 18% of this recommendation. Therefore, the PV prediction method can be considered excellent as its total error was of magnitude 25% or less (McFarren, Lishka & Parker, 1970).

Regarding AI, the best PLS model was obtained when the NIR spectra were pre-processed 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 with 7 latent variables), Table 3 and Figure 3. As indicated in Table 3, the SEP and $RMSE_P$ differences were not significant which could be taken as an indication that the prediction precision of the PLS regression was high (Amodio, Ceglia, Chaudhrya, Piazzolla & Colellia, 2017), although R^2 was low. Similar $RMSE_P$ values (0.35%) were reported by Mba, Adewale, Dumont & Ngadi (2014) in palm and canola oils with NIR spectra pre-processed using SG first derivative, but their R^2 was much greater (0.99). Better results were reported by Armenta and La Guardia (2007) in olive oil (RMSEP = 0.083% and R2 = 0.99); once again, the difference between food matrixes must be taken into consideration. Although the R^2 values were below 0.70, considering the AI level of 0.5% as the maximum quality limit of macadamia nuts (SAMAC, 2015), the SEP value represented 28% of this limit. Consequently, the AI prediction as an analytical method can be considered acceptable as its total error was of the magnitude of 50 % or less (McFarren, Lishka & Parker, 1970).

The moisture prediction results (Table 3, Figure 4) indicate that the best calibration models for moisture content of unshelled nuts were obtained using SG + 2nd derivative (Rc^2 = 0.66; RMSEc

= 2.33%). Better results were reported by Guthrie, Greensill, Bowden & Walsh (2005) who found moisture content prediction models with RMSEcv of 0.11% and Rc^2 of 0.79. Their results were improved by using ground nuts (RMSEcv = 0.06% Rc^2 = 0.95), and these differences could be due to the lack of moisture homogeneity within our intact kernels; grinding should have reduced this variation. Sumdaram, Kandala, Holser, Butts & Windham. (2010) also reported better results for in shell peanuts using NIRS (RMSEp = 1.38% and R^2c = 0.98), but the moisture content range was much higher (6.18 - 21.69 %) and should be taken into consideration.

4. Conclusion

Cultivars had little effect on the oxidative stability of intact unshelled macadamia nuts based on PV and AI. On the other hand, the drying process affected mostly the AI as the PV did not present any modifications during drying.

The NIR spectra were similar between cultivars, but the absorption bands changed during the drying process and the characteristic oil bands could be seen when the MSC, SNV, and SG smoothing derivatives were applied.

The PLS calibration models for PV and AI were considered useful since the error is lower of the quality limits. Therefore, NIRS can be used to assess the oxidative stability of intact macadamia nuts, but further investigation must be done to address other sources of variability to improve the robustness of the prediction models.

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*These references were fundamental for the discussion of the article, since they deal with works similar to the above, using macadamia nuts, near infrared spectroscopy combined with chemometrics and oxidative evaluation of nuts, clearly exposing the quality parameters of macadamia nuts.

Tables

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

[a]SD = standard deviation. *from the total of 240 samples, 20 were lost during oil extraction which corresponds a total of 220 samples.

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437

438 **Table 2.** Moisture, dry matter, peroxide value, and acidity index of unshelled macadamia
439 (*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.

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446 **Table 3.** Statistical parameters for the optimal prediction models for peroxide value, acidity index,
447 and moisture of unshelled macadamia (*Macadamia integrifolia* Maiden & Betche) from different
448 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

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

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455 **Figures**

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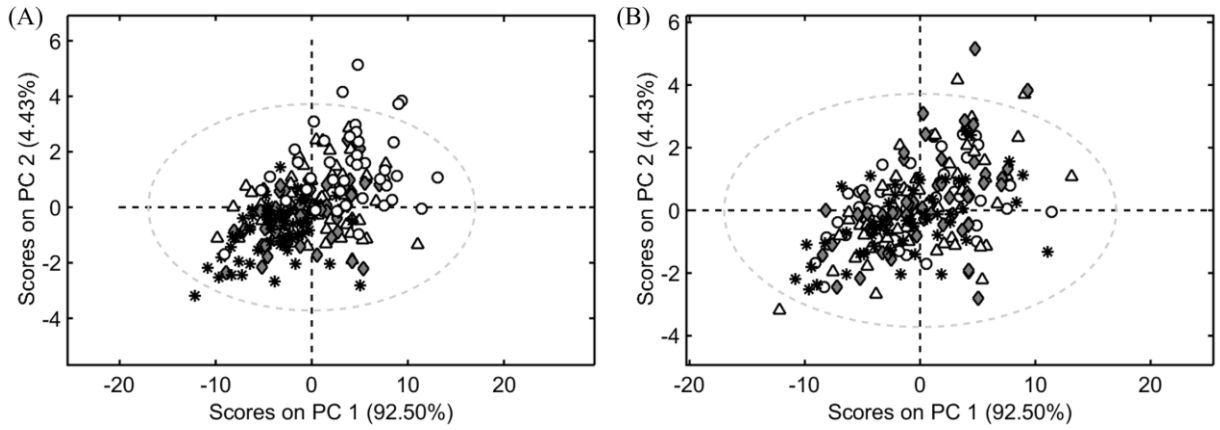


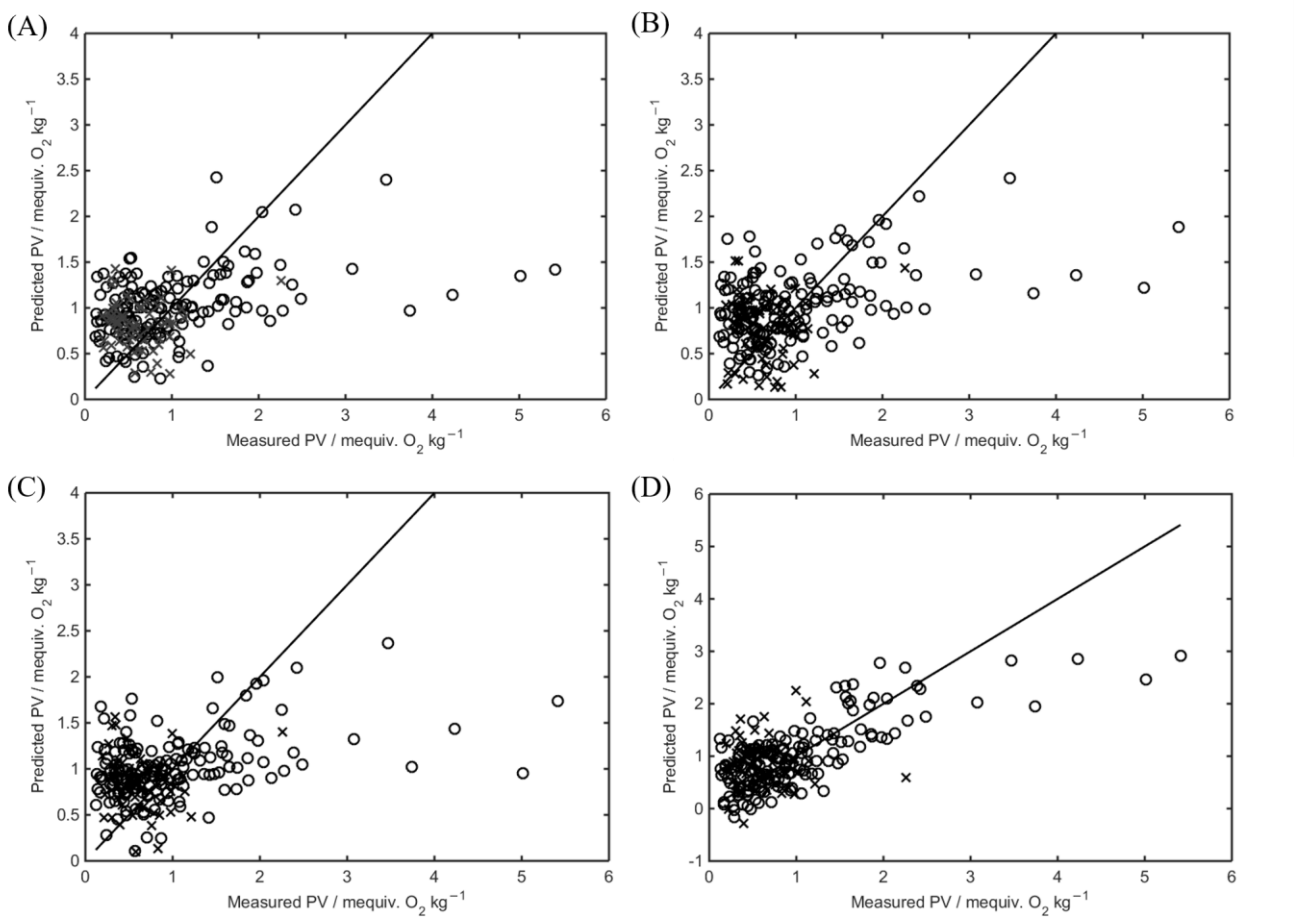
459

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

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

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

463 HAES 340 [○]).



464

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

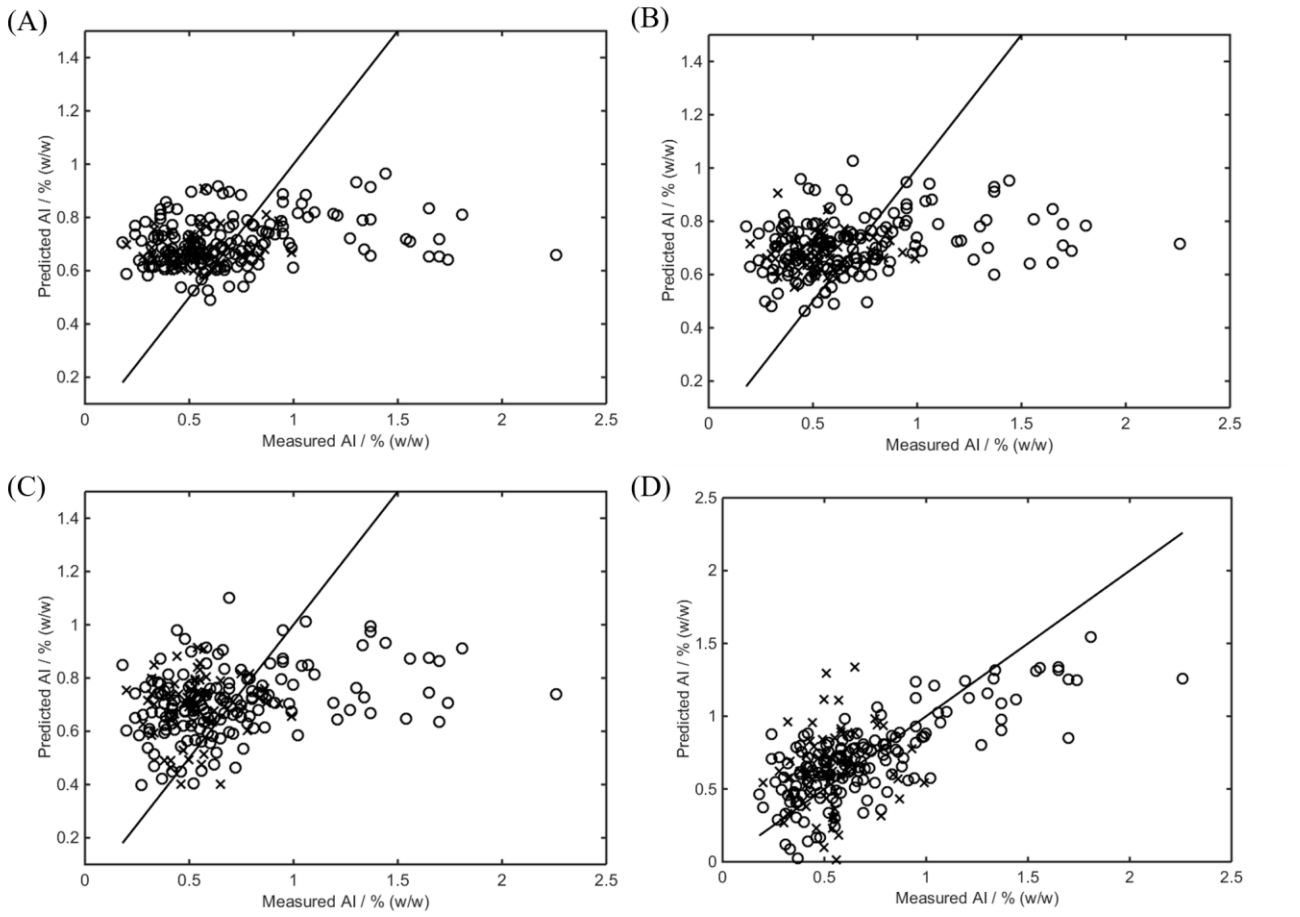
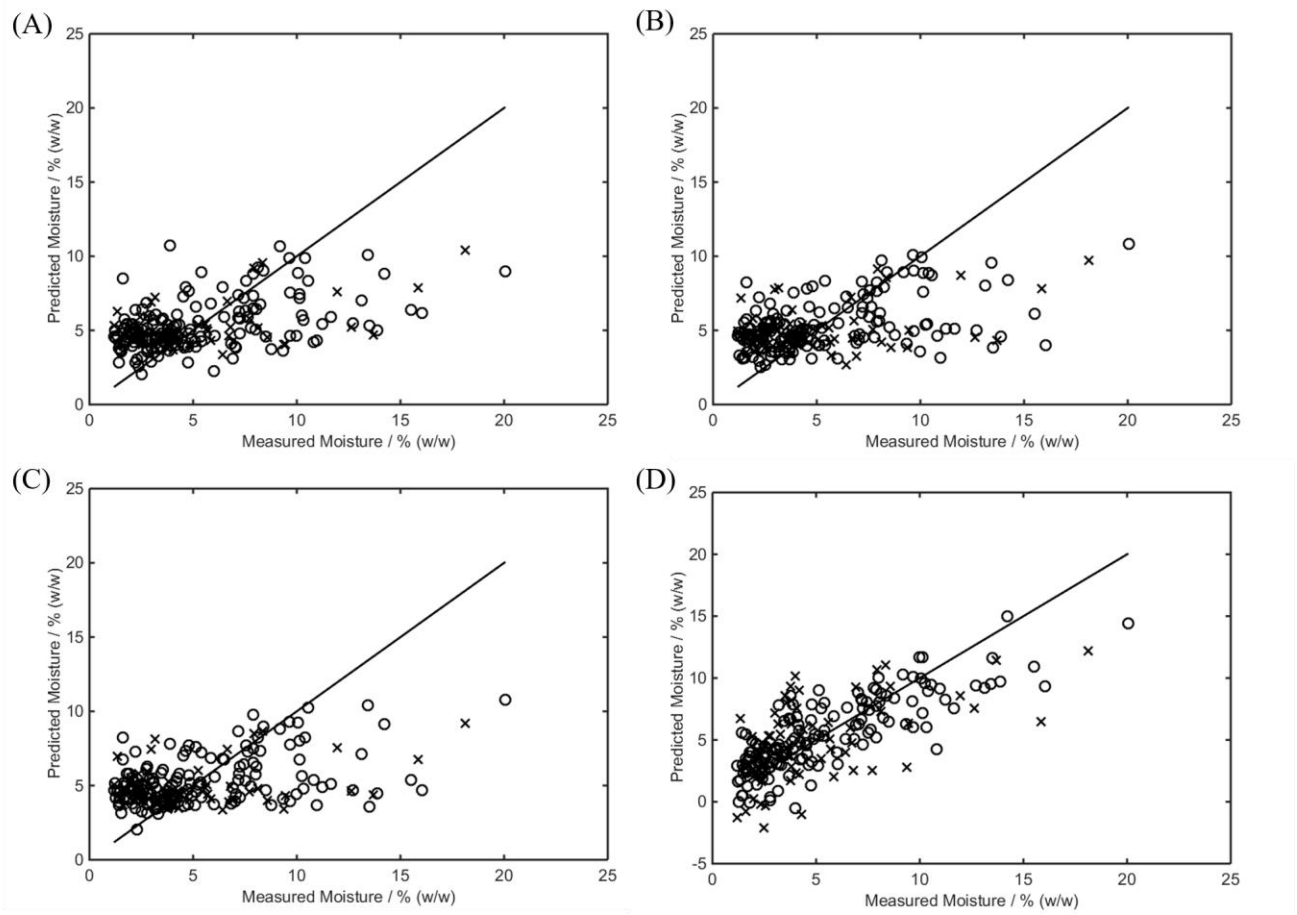


Figure 3. Measured *versus* predicted acidity index (AI) for the calibration (o) and validation (x) sets using PLS regression with the following pre-processing: (A) raw spectra; (B) SG+SNV; (C) SG+MSC+baseline; (D) SG+2nd derivative. SG = Savitzky-Golay smoothing; SNV = standard normal variate; MSC =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



474

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