Title

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Journal

ACS Omega, 3(11)

ISSN

2470-1343

Authors

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Publication Date

2018-11-30

DOI

10.1021/acsomega.8b02491

Peer reviewed

ACS OMEGA

Article

Cite This: ACS Omega 2018, 3, 16081−16088

http://pubs.acs.org/journal/acsodf

Near-Infrared (NIR) Spectrometry as a Fast and Reliable Tool for Fat and Moisture Analyses in Olives

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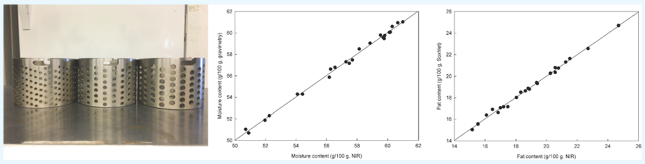
ABSTRACT: The evaluation of fat and moisture contents for olive fruits is crucial for both olive growers and olive oil processors. Reference methods, such as Soxhlet extraction, used for fat content determination in olive fruits are time- and solvent- consuming and labor intensive. Near-infrared (NIR) spectroscopy is proposed as a solution toward rapid and nondestructive analyses of olive fruit fat and moisture contents. In the present work, comparative studies of the fat and moisture quantification methods were performed on four cultivars (Arbosana, Arbequina, Chiquitita, and Koroneiki) during six different harvesting time points to determine the potential of NIR as an alternative methodology. The impact of olive paste crushing degree on NIR performance was also investigated using three different grid sizes (4, 6, and 8 mm) on a hammer mill, in addition to a blade crusher. Results indicate a satisfactory correlation between the reference Soxhlet and NIR methods with $R^2 = 0.995$. A comparison study of moisture content was also done on NIR and the use of conventional oven with the R^2 value of 0.995. The crushing blade produced higher values in both moisture and fat contents in comparison to the hammer mill. The evaluation indicates that when building a chemometric model, all crush sizes and blade sizes should be represented in the model for highest accuracy.



1. INTRODUCTION

As olive oil began to gain popularity in the consumer market, a growing emphasis on optimizing the olive oil extraction processes and the quality of the oil appeared. Virgin olive oil is extracted from the fruit of *Olea Europaea* L. by crushing the olive fruit, mixing the resulting paste in a malaxer, and separating the oil by centrifugation. The quality of olive oil relies on the quality of the olive fruit.[1] Fat and moisture content determinations are routine measurements performed to establish optimum harvest time since they have effects on the efficiency of oil extraction and the quality of oil.

At commercial laboratories, traditional analytical techniques, like gravimetry with conventional oven and Soxhlet extraction, are commonly used. Although these techniques are widely accepted as reference methods, the determination of olive moisture content is often labor intensive and time consuming. Moreover, not only does the Soxhlet extraction often require long extraction time, but is highly solvent consuming and impractical. Near-infrared (NIR) spectroscopy is an alternative solution toward a rapid and safe analysis of olive fruits. NIR requires little sample preparation and is consider safer than traditional wet chemistry analytical techniques such that it does not require any hazardous chemicals. However, expertize in chemometric analysis is often a requirement, especially to offer calibrations that are robust and reliable. Although a model needs to be established and optimized with specific olive fruit samples, once the calibration model is build, it can produce results comparable to reference methods.

Literature shows the feasibility of NIR as an alternative method to determine fat and moisture contents[2−9] as well as to quantify parameters associated with the quality of olives, such as fatty acid composition and characterization of geographic origin.[10−13]

Furthermore, NIR has recently been shown to be applicable to process monitoring to ensure the quality of the olive products, including olive oils, olive pomace, and olive fruits.[11,14−17] A study by Bendini et al. demonstrated a satisfactory correlation for moisture content and fatty acid using an at-line NIR instrument with the coupling of partial least squares (PLS) regression models.[11] The application of NIR to on-line control quality and characterization of virgin olive oil where acidity value ($R^2 = 0.999$), bitter taste ($R^2 = 0.936$), fatty acid oleic ($R^2 = 0.998$), and fatty acid linoleic ($R^2 = 0.992$) were study.[14] Mailer et al. developed NIR calibrations for various quality parameters in olive oil, such as free fatty acid, peroxide value, polyphenol content, induction time, chlorophyll, and fatty acid, which allows routine screening of olive oil.[15] Mesa et al. showed the potential ability for NIR to analyze oil and moisture in two-phase olive pomace.[17]

NIR has demonstrated many advantages, but the cost of installment may limit the accessibility of NIR to many industries. Nevertheless, the integration of in-process monitoring may prevent olive processors from investing excess time

Received: September 23, 2018
Accepted: November 8, 2018
Published: November 28, 2018

DOI: 10.1021/acsomega.8b02491

Figure 1. (a) Hammer mill crusher; (b) three different grid sizes (4, 6, and 8 mm) used with the hammer mill; (c) blade crusher. Photograph courtesy of Chiaohwei Lee. Copyright 2017.

and effort into producing dissatisfying products. By integrating NIR into an industrial process, processors are able to obtain information in sufficient time to make any adjustments to the process. This is made possible by the near instantaneous analysis of the sample by NIR.

Although NIR has been suggested as an alternative methodology for moisture and fat determinations of olive fruit,[2−9] the reliability of NIR to displace the usage of traditional analytical techniques has yet to be tested. Furthermore, a standardized method for fat and moisture content determinations using NIR should be developed to avoid inconsistencies in results. Even though NIR requires little sample preparation, the analysis is affected by the physical properties of the sample, such as size and shape, packing of the sample, surface and color.[18] In this study, in addition to different cultivars and harvesting time points, the particle size of the olive paste will be taken into consideration to observe the relationship between crushing degree and precision of NIR to provide fat and moisture contents of the intact olives. By understanding the relationship between the physical properties of the sample and NIR, actions can be taken to ameliorate fat and moisture content determinations using NIR.

The crushing profile of the fruit may impact the results obtained by NIR due to the parameters that involves the surface area of the sample.[18,19] Although literature that relates the sample particle size to NIR reflectance spectrometry is scarce, the relationship between particle size and NIR reflectance has been done on powdered substances, like pharmaceuticals.[19] Reflection spectrometry is the study of light reflected from the surface area of the samples rather than the amount of light transmitted through the sample. The reflectance of the samples is dependent on the concentration, absorptivity, and the scattering coefficient according to the Kubelka−Munk theory. The NIR spectrum is affected by the chemical composition as well as the physical properties, such as the particle sizes and surface area.[18,19] Therefore, it is important to identify the relationship between the characteristics of the sample and NIR to standardize and optimize olive fat and moisture measurements using NIR.

There are two main objectives in this study: (1) to determine the accuracy and precision of NIR screening on fat and moisture contents to traditional analytical techniques on four cultivars at different harvesting time points; (2) to investigate the influence of paste texture on NIR screening using three grid sizes and two different crushers, as shown in Figure 1.

2. RESULTS AND DISCUSSION

2.1. Comparison Study of NIR and Reference Methods.

The statistics of moisture and fat models is detailed in Table 1. The ranges of both the moisture and fat contents are relatively small, ranging from 15.05 to 24.7 for moisture content and 50.69−61.03 for fat content. The correlation coefficient and standard of error are used to show the adequacy of the model. NIR predictive model obtained from PLS showed satisfactory performance as demonstrated by the R^2 values of 98.0 and 97.8 from the calibration of moisture and fat contents, respectively. The standard error of prediction (SEP) values of moisture and fat contents from the validation model are similar to the standard error of calibration (SEC) values of the calibration model, indicating that the calibration equation can produce results with great precision.

Table 1. Statistics of the Moisture Content and Fat Content Models

moisture content (g/100 g)					
validation			calibration		
range (g/100 g)	R^{2a}	SEP[b] (g/100 g)	range (g/100 g)	R^{2a}	SEC[c] (g/100 g)
15.05−24.7	98.0	0.34	15.05−24.7	98.9	0.29
fat content (g/100 g)					
validation			calibration		
range (g/100 g)	R^{2a}	SEP[b] (g/100 g)	range (g/100 g)	R^{2a}	SEC[c] (g/100 g)
50.69−61.03	97.8	0.45	50.69−61.03	99.4	0.31

[a] Coefficient of calibration/validation. [b] Standard error of prediction. [c] Standard error of calibration.

The regression analysis indicates a good correlation (R^2 = 0.995) between the reference method and NIR for moisture content when the triplicate runs average values are graphed (Figure 2). Additionally, the 95% confidence intervals for *y*-intercept and slope are (−1.983, 1.704) and (0.971, 1.035), respectively.

Figure 3 shows the linear regression for the triplicate runs average values of the reference method and NIR for fat content. The regression analysis yielded a good correlation coefficient (R^2 = 0.995). The 95% confidence intervals for the *y*-intercept are (−0.466, 0.778) and (0.960, 1.025) for the slope.

The correlation of fat content measured with reference method has shown in other literature to be less satisfactory

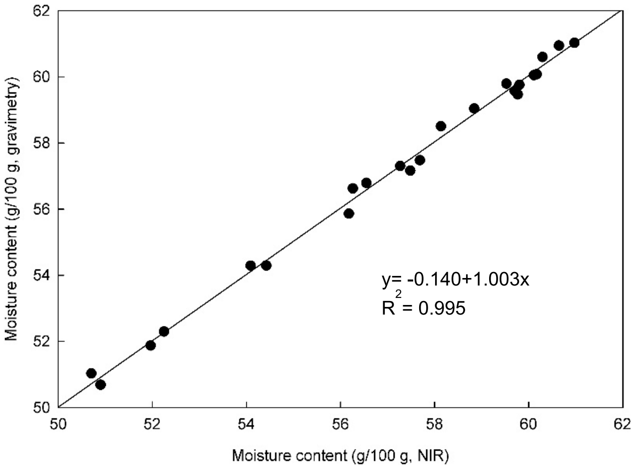


Figure 2. Linear regression analysis of moisture content measured by gravimetric analysis and NIR screening. The average values of the triplicate runs of the reference method and NIR validation are graphed.

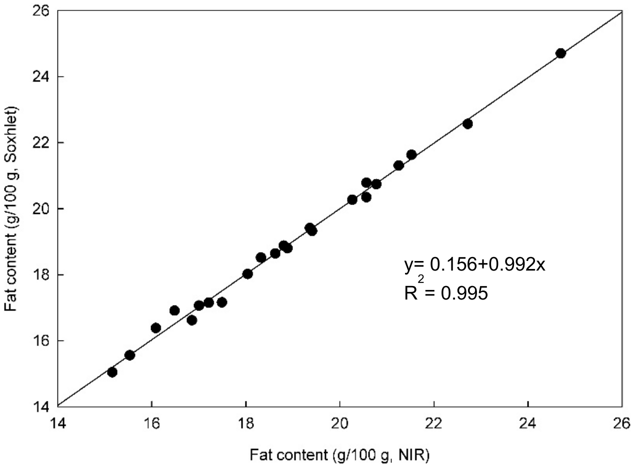


Figure 3. Linear regression analysis of fat content measured by Soxhlet extraction and NIR screening. The average values of the triplicate runs of the reference method and NIR validation are graphed.

than moisture content measured with gravimetry.[5,11,20] Cayuela et al. obtained regression models by partial least squares (PLS) and NIR for the analyses of fat (R^2 = 0.86) and moisture content (R^2 = 0.91) with minimal predictive errors.[5] Bendini et al. investigated the feasibility of integrating NIR in industrial olive mill by collecting the moisture and fat contents of intact olives using NIR and PLS regression. It is concluded that NIR provided satisfactory correlation for moisture content, but the fat content predicted by NIR was poor with R^2 value of 0.605.[11] Jimenez et al. conducted a similar experiment using NIR reflectance spectroscopy to measure fat and moisture contents in intact fruit after treating with a hammer mill crusher and reported standard errors of 0.811 and 0.928 for fat and moisture contents, respectively.[20] In this study, both fat and moisture contents demonstrated satisfactory correlation with R^2 of 0.995. Since this study included 23 samples of olives, a future study with more samples should be used to build a robust model to validate the results found.

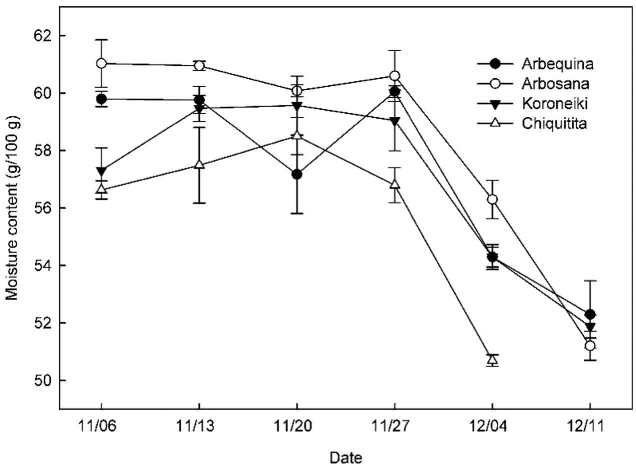


Figure 4. Moisture contents for Arbequina, Arbosana, Koroneiki, and Chiquitita cultivars determined by gravimetry throughout the harvesting season.

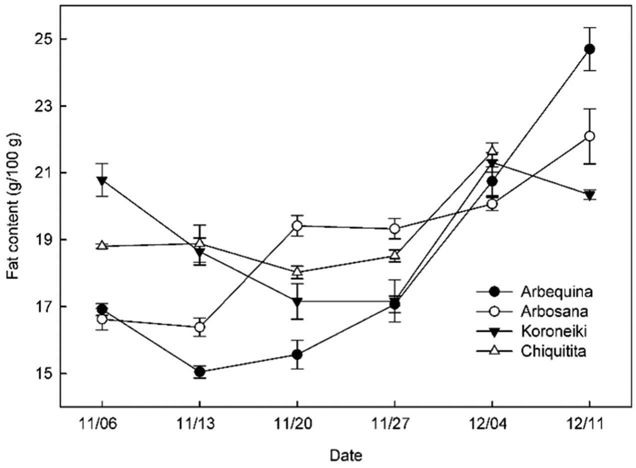


Figure 5. Fat contents for Arbequina, Arbosana, Koroneiki, and Chiquitita cultivars determined by Soxhlet extraction throughout the harvesting season.

2.2. Maturity. Figures 4 and 5 show the changes in moisture and fat contents (in dry basis) obtained by the reference method for the four studied cultivars from November 6 to December 11. Moisture content presented an overall decreasing trend with time, which is consistent with literature.[1,21] All cultivars except for Arbequina showed little changes in moisture content in the beginning of the study period until December 4 where all four cultivars demonstrate a drastic decrease in moisture content. Arbequina began the harvesting season with very little changes (59.8 ± 0.3% on November 6 and 58.8 ± 0.5% on November 13). However, a significant decrease on November 20 (57.2 ± 1.4%) can be observed. The moisture content of Arbequina then increased on November 27 (60.1 ± 0.2%) and began to decrease similarly to other cultivars with 54.3 ± 0.4% on December 4 and 52.3 ± 0.7% on December 11. Of all of the cultivars, Koroneiki has the lowest percent moisture (56.6 ± 0.3%) in the beginning of the harvesting period with the most dramatic decreasing trend on December 4 (54.3 ± 0.3%). Unfortunately, the time point on December 11 for Koroneiki is missing. Arbosana showed the highest moisture content in and

Table 2. Fat and Moisture Contents of Olive Paste with Different Particle Sizes Determined by NIR Screening[a]

harvest time	cultivar	maturity index	type of crusher	moisture content	fat content
Nov 6	Arbosana	0.6	4 HM	61.2 ± 0.32^{a}	16.80 ± 0.18$^{c.b}$
			6 HM	60.97 ± 0.15^{a}	16.86 ± 0.24^{b}
			8 HM	outlier	outlier
			BC	61.16 ± 0.71^{a}	17.37 ± 0.17^{a}
	Arbequina	0.7	4 HM	59.19 ± 0.20^{a}	16.66 ± 0.37^{a,c}
			6 HM	59.53 ± 0.30^{a}	16.49 ± 0.13^{b,c}
			8 HM	outlier	outlier
			BC	60.06 ± 0.58^{a}	17.23 ± 0.31^{a}
	Chiquitita	0.9	4 HM	57.91 ± 0.59^{a}	18.52 ± 0.52^{b}
			6 HM	57.27 ± 0.06^{a}	20.56 ± 0.37^{a}
			8 HM	outlier	outlier
			BC	58.35 ± 0.50^{a}	19.85 ± 0.48^{a}
	Koroneiki	0.4	4 HM	56.21 ± 0.50^{a}	18.82 ± 0.23^{a}
			6 HM	56.26 ± 0.36^{a}	18.88 ± 0.10^{a}
			8 HM	55.76 ± 0.49^{a}	18.97 ± 0.14^{a}
			BC	56.20 ± 0.57^{a}	19.51 ± 0.47^{a}
Nov 13	Arbosana	0.9	4 HM	59.31 ± 0.56^{a,b}	16.13 ± 0.58^{a}
			6 HM	60.65 ± 0.30^{c}	16.09 ± 0.47^{a}
			8 HM	outlier	outlier
			BC	59.32 ± 0.38^{a,d}	17.06 ± 0.23^{a}
	Arbequina	1.0	4 HM	59.48 ± 0.70^{c,d}	16.82 ± 0.03^{c}
			6 HM	59.80 ± 0.27^{b,d}	15.16 ± 0.30^{b}
			8 HM	60.69^{a,b,c}	16.64 ± 0.82^{a}
			BC	61.19 ± 0.34^{a}	15.32 ± 0.57^{a,b}
	Chiquitita	0.9	4 HM	59.46 ± 0.61^{a}	17.53 ± 0.65^{b,c,d}
			6 HM	59.77 ± 0.43^{a}	18.63 ± 0.28^{a,d}
			8 HM	58.55 ± 0.70^{a}	16.79 ± 0.44^{b}
			BC	58.91 ± 0.55^{a}	18.69 ± 0.07^{a}
	Koroneiki	0.5	4 HM	57.47 ± 0.97^{a}	17.56 ± 0.33^{b,c}
			6 HM	57.69 ± 0.14^{a}	18.81 ± 0.23^{a}
			8 HM	56.66^{a}	16.36^{b}
			BC	58.80^{a}	18.59 ± 0.42^{a,c}
Nov 20	Arbosana	1.0	4 HM	60.17 ± 0.61^{a}	19.36 ± 0.46^{b}
			6 HM	59.06 ± 0.37^{a}	18.66 ± 0.41^{a,b}
			8 HM	59.78 ± 0.44^{a}	17.85 ± 0.47^{a,c}
			BC	60.22 ± 0.64^{a}	17.92^{a,b}
	Arbequina	1.0	4 HM	57.48 ± 0.28^{b}	15.53 ± 0.33^{b}
			6 HM	59.89 ± 0.66^{a,c}	17.51 ± 0.60^{a}
			8 HM	60.14 ± 0.59^{a}	17.46 ± 0.72^{a}
			BC	59.35 ± 0.86^{a}	16.43 ± 0.32^{a,b}
	Chiquitita	1.2	4 HM	59.70 ± 0.11^{a}	17.21 ± 0.27^{b}
			6 HM	58.66 ± 0.21^{b,c}	17.20 ± 0.40^{b}
			8 HM	59.29 ± 0.30^{a,c}	16.22 ± 0.45^{a}
			BC	59.41 ± 0.34^{a}	16.90 ± 0.33^{a,b}
	Koroneiki	0.9	4 HM	58.13 ± 0.52^{a}	18.04 ± 0.20^{b}
			6 HM	57.57 ± 0.57^{a}	17.72 ± 0.40^{a,b}
			8 HM	58.15 ± 0.99^{a}	17.40 ± 0.19^{a,b}
			BC	58.94 ± 0.63^{a}	17.29 ± 0.06^{a}
Nov 27	Arbosana	1.0	4 HM	60.29 ± 0.31^{a}	19.41 ± 0.10^{c}
			6 HM	59.81 ± 0.63^{a}	18.64 ± 0.14^{a}
			8 HM	59.62^{a}	18.53^{a}
			BC	59.99 ± 0.73^{a}	20.00 ± 0.23^{b}
	Arbequina	1.2	4 HM	60.11 ± 0.23^{a}	17.01 ± 0.22^{a}
			6 HM	59.01 ± 0.64^{a}	17.11 ± 0.68^{a}
			8 HM	59.53 ± 0.78^{a}	17.62 ± 0.38^{a}
			BC	58.94 ± 0.76^{a}	17.87 ± 0.60^{a}
	Chiquitita	1.4	4 HM	58.84 ± 0.17^{a}	17.49 ± 0.26^{b,c,d}
			6 HM	57.93 ± 0.92^{a}	17.49 ± 0.56^{a,d}
			8 HM	58.23 ± 0.58^{a}	17.85^{a,c}
			BC	57.81 ± 0.90^{a}	18.70 ± 0.34^{a}
	Koroneiki	0.7	4 HM	56.55 ± 0.46^{a}	18.32 ± 0.04^{a}

Table 2. continued

harvest time	cultivar	maturity index	type of crusher	moisture content	fat content
			6 HM	55.54 ± 0.58^{a}	17.39 ± 0.49^{a}
			8 HM	56.14 ± 0.53^{a}	17.67 ± 1.10^{a}
			BC	56.60 ± 0.71^{a}	18.69 ± 0.31^{a}
Dec 4	Arbosana	1.4	4 HM	56.18 ± 0.38^{a}	20.26 ± 0.17^{a}
			6 HM	57.78 ± 0.32^{a}	20.00 ± 0.64^{a}
			8 HM	56.66 ± 0.96^{a}	19.50 ± 1.37^{a}
			BC	57.09 ± 0.77^{a}	20.13 ± 0.21^{a}
	Arbequina	1.5	4 HM	54.42 ± 0.25^{a}	20.78 ± 0.05^{a}
			6 HM	55.30 ± 0.91^{a}	20.55 ± 1.01^{a}
			8 HM	55.65 ± 1.19^{a}	20.18^{a}
			BC	55.50 ± 0.43^{a}	20.82 ± 0.48^{a}
	Chiquitita	1.3	4 HM	54.09 ± 0.18^{a}	21.25 ± 0.05^{a}
			6 HM	53.55 ± 0.92^{a}	20.34 ± 0.65^{a}
			8 HM	53.80^{a}	21.02^{a}
			BC	53.50 ± 0.64^{a}	20.32 ± 0.42^{a}
	Koroneiki	1.0	4 HM	50.90 ± 0.46^{a}	21.52 ± 0.38^{a}
			6 HM	50.97 ± 0.08^{a}	21.44 ± 0.42^{a}
			8 HM	outlier	22.87 ± 0.33^{b}
			BC	50.33 ± 0.59^{a}	20.91 ± 0.35^{a}
Dec 11	Arbosana	1.5	4 HM	50.83 ± 0.55^{a}	22.70 ± 0.16^{a}
			6 HM	51.49 ± 0.94^{a}	20.94^{b}
			8 HM	51.73 ± 0.53^{a}	outlier
			BC	51.91 ± 0.05^{a}	23.28 ± 0.37^{a}
	Arbequina	3.0	4 HM	52.25 ± 0.20^{a}	24.70 ± 0.39^{a}
			6 HM	52.05 ± 0.03^{a}	25.03 ± 0.78^{a}
			8 HM	52.86 ± 0.25^{b}	outlier
			BC	52.31 ± 0.20^{a}	23.93 ± 0.28^{a}
	Chiquitita	2.0	4 HM	51.97 ± 0.06^{a}	20.57 ± 0.29^{a}
			6 HM	52.58 ± 0.19^{a}	outlier
			8 HM	52.81^{a}	outlier
			BC	52.39 ± 1.00^{a}	22.10 ± 1.22^{a}
	Koroneiki	2.5	4 HM	49.44 ± 0.50^{a}	20.75 ± 0.12^{a}
			6 HM	49.54 ± 0.65^{a}	20.90 ± 0.34^{a}
			8 HM	49.65 ± 0.50^{a}	20.85^{a}
			BC	50.78 ± 0.44^{a}	21.93^{a}

[a] Values are expressed as mean ± standard deviation; (a, b, c,...) indicates significant differences according to Tukey test; 4 HM, 6 HM, 8 HM represent 4, 6, 8 mm hammer mill and BC represents blade crusher.

throughout the harvesting period (61.0 ± 0.8, 60.9 ± 0.2, 60.1 ± 0.2, 60.6 ± 0.9, 56.3 ± 0.7% on November 6, 13, 20, 27, and December 4, consecutively) until December 11, where it drastically decreased to the lowest moisture content of the four cultivars (51.2 ± 0.5%). The moisture content of Chiquitita increased on November 13 (59.5 ± 0.5%) and remained steady until December 4 (54.3 ± 0.3%) where a prominent decreasing trend is observed. The variability of the moisture content as the fruit matures is not uncommon considering that moisture content is highly variable across maturation period as olives tend to absorb more moisture when it is abundant, but release water when it is drier. The moisture content of the olive fruit is dependent on cultivars and environmental conditions,[21,22] therefore some deviation overtime is expected.

Fat content showed an increasing trend with time, again consistent with what has been shown literature.[1,23] The most dramatic increase in fat content of the four cultivars is observed on December 4. Chiquitita had little overall changes during the studied time period, though some decreases earlier in the season and a large increase during November 27 (17.16 ± 0.6%) and December 4 (21.31 ± 0.3%) were observed. Since its fat content was among the highest at the starting period, it is possible that the fat content had reached its peak for Chiquitita. Koroneiki and Arbequina experienced little overall changes during the period of November 6 to November 27, however, a significant increase was observed for Arbequina after November 27 (data missing for Koroneiki). The fat contents for Koroneiki were 18.8 ± 0.1, 18.9 ± 0.6, 18.0 ± 0.2, 18.5 ± 0.2, 21.3 ± 0.3% on November 6, 13, 20, 27, and December 4. Arbosana had the most consistent increasing trend comparing to other studied cultivars with drastic increasing fat content on November 13 (16.4 ± 0.3%). The increasing trend of fat content collected from all four cultivars is parallel to results found in literature. The accumulation of fat content in olive fruits is slightly influenced by the maturity as well as the climatic conditions of the olives.[23] Therefore, the overall increasing trend of fat content in this study is not solely dictated by the ripeness of the fruit. The inconsistency found during the study period is an indicator that the climatic conditions of the olive fruits may have impacted the fat content.

To assess how accuracy changes with harvest time, a difference in values obtained by the reference methods and NIR was calculated. All fruits harvested at the same time point for the reference methods and NIR were averaged to determine the differences in the mean values, which can be used as a marker of how closely related is the value predicted by NIR to the reference methods. The mean differences of both moisture and fat contents are small, demonstrating mean differences within the range of ±0.2%. There were no significant trends observed across the study period for both fat and moisture contents. The standard deviations for both moisture and fat contents are big; therefore, statistically, there are no differences in the accuracy of NIR across the study period.

2.3. Cultivars.

Moisture content slightly varied among different cultivars. Three of the four cultivars showed a steady range of moisture content in the month of November except for Arbequina. Arbosana ranged between 51.2 ± 0.5 and 61.90 ± 0.2%, whereas Koroneiki had range of 50.7 ± 0.2 and 58.5 ± 0.7%. The moisture content of Chiquitita was in the range of 51.9 ± 0.4 and 59.6 ± 1.0%. All four of the cultivars drastically decreased in moisture content in the beginning of December, reaching a minimum on December 11. Arbequina had a maximum of 60.17 ± 0.2% and a minimum of 52.3 ± 1.2%. Different from the other cultivars, Arbequina deviated from the general decreasing trend for moisture content with a drastic decrease observed on November 20 (57.2 ± 1.4%), followed by an increase in moisture content on November 27 (60.1 ± 0.2%), which then continued to drop for the remaining study period. Considering that there are many factors that could influence the moisture content of olives, it is difficult to determine the reason behind the inconsistency observed for Arbequina. However, it is possible that the moisture content fluctuates due to external reasons, such as the sensitivity and adaptability of fruits to the climate.[22,23]

Fat content of all four cultivars increased overtime during the study period. The ranges of fat content obtained by the reference method are as follows: Arbequina (15.05 ± 0.2 to 24.70 ± 0.6%); Arbosana (16.38 ± 0.3 to 22.56 ± 0.8%); Koroneiki (18.02 ± 0.2 to 21.63 ± 0.3%); Chiquitita (17.15 ± 0.5 to 21.31 ± 0.3%). The maximum fat contents of Arbequina and Arbosana were observed on December 11, whereas the maximum fat contents of Koroneiki and Chiquitita were observed on December 4. The percent fat for Chiquitita had shown to decrease on November 11, whereas Arbequina, Arbosana, and Koroneiki continued to increase. As seen in Figure 3b, the amounts of percent fat for each cultivar were slightly different in the early stages, and each cultivar had increased in varying amounts. However, as the fruit matured, the fat content recovered from each cultivar became greater. The highest increase in fat content was for Arbequina, which peaked toward the end of our experiment in mid-December.

The differences between the prediction provided by NIR and the reference methods for different cultivars were also calculated. There were no significant differences for both moisture and fat contents, indicating that the accuracy of NIR does not vary between cultivars. However, NIR has demonstrated a greater ability to predict fat content (within the range of −0.05−0.06) than moisture content (within the range of −0.02−0.11). The predictions provided by NIR closely represent the reference methods for fat content, demonstrated with the small mean differences values, in comparison to moisture content. This differs from previous literature, as NIR was found to be more effective for moisture content than fat content.[5,11,20] This may be explained by the difficulty to analyze spectra within the range of moisture content due to the overlapping of the water fingerprint region. Two broad peaks around 1440 and 1930 nm, generally caused by water, may cause complications during NIR analysis.[24]

2.4. Crushers and Grid Sizes.

Particle size distribution of the sample is a crucial parameter of NIR reflectance spectrum.[18,19] The crushing profile of the olive fruit has shown to be one of the parameters that could influence the accuracy of NIR screening.[18] The scattering and reflection of the light on different textures and surface areas of the sample can influence the screening of NIR.[19] Therefore, the effects of the particle characteristics of the olive paste and crushing method are taken into account for this experiment.

Table 2 shows the fat and moisture contents of the different crushing profiles of NIR screening. Some data points have been eliminated as outliers because the spectra do not match the calibration spectra closely enough to offer a reliable prediction. The ability for NIR to identify spectra that are outliers indicate that NIR is able to distinguish spectra that are widely different from the spectra provided by the calibration model. Since the calibration model is built based on the 4 mm hammer mill samples, olive samples crushed with 6, 8 mm hammer mill and blade crusher were purely blind predictions. The calibration model was too specific to analyze olive samples crushed with 6, 8 mm hammer mill and blade crusher due to these spectra being too different from the spectra that are contained in the calibration.

Despite some data points eliminated, trends among 4, 6, 8 mm grid sizes were observed. Because the used model is based solely on 4 mm grid size crushed fruits, the fat and moisture contents of 6, 8 mm grid sizes on the hammer mill and the blade crusher are predictions made by the NIR calibration model. Olives crushed with 4 mm grid sizes on the laboratory hammer mill have generally demonstrated higher moisture content yield in comparison to olives crushed with 8 mm grid. The diffusion component of reflected energy depends on the physical nature of the sample because increasing surface area enables more particles to spread through the sample.[19] The Kubelka−Munk model can be used to describe the importance of light propagation through an inhomogeneous mixture and a homogenous mixture, which can create different scatterings of lights and therefore influencing the NIR result.[25] The increasing moisture results of the NIR with decreasing grid size could partly be explained by this, but is more likely a consequence of the grid sizes not being represented in the calibration model. The fat content screened by NIR showed unexpected results with majority of the samples demonstrating a lower fat yield in response to 4 mm grid. The inconsistencies observed for fat content are likely a result of the calibration model having only 4 mm grid size data. Since a majority of the 8 mm hammer mill olive paste was predicted as outliers, this is a clear indication that incorporation of data for each blade size to be analyzed is crucial for a robust and reliable calibration.

The crushing treatment of the fruit has proven to influence moisture and fat contents. Inarejos-García et al. demonstrated that olives crushed with a hammer mill resulted in higher fat content.[26] The authors hypothesized that a high speed hammer mill may cause a more complete breakage of fruit tissue due to the more aggressive crushing mechanism.[26] In this study, the differences in crushing methods have shown to be mainly insignificant by analysis of variance (ANOVA) statistical analysis. Among the small sample size that showed significance in different crushing methods, the blade crusher is observed to provide a higher fat and moisture content than a laboratory mill crusher when NIR is used. This indicates that the crushing methods are one of the factors, which can affect the NIR measurement. Considering the great differences in the fat and moisture contents between the hammer mill and the blade crusher, other parameters, like the speed of rotation and the temperature of the system, may have impacted the total yield.[26,27]

3. CONCLUSIONS

Near-infrared spectrometry has potential as an alternative method for fat and moisture determinations in olives. The correlations between NIR and the reference methods have shown to be very good for both moisture and fat contents. The sample treatment of the olive fruit, such as using different grid sizes and crushers, can influence the results obtained by NIR. Due to the small sample size obtained from this study, the influences of different particle sizes and crushing methods on NIR are inconclusive. In this study, olives crushed with 4 mm grid size on the hammer mill resulted in higher moisture content, but lower fat content in comparison to olives crushed with 8, 6 mm grid size on the hammer mill and blade crusher. The calibration model was built using only olives crushed with 4 mm grid size on the hammer mill. Therefore, this may influence the predictions made for olives crushed with 6, 8 mm grid sizes on the hammer mill and the blade crusher. However, due to the good correlation between NIR and the reference methods, it can be concluded that if the NIR samples were prepared similarly to the sample used for the calibration model, a more robust calibration model can be built, providing a more accurate result for both fat and moisture contents. A calibration model built using different grid sizes and crushing methods should be integrated to further confirm this conclusion. Furthermore, it is necessary to conduct cross-lab ring tests, including larger sample sizes, to further validate and improve the results found in this study.

4. MATERIALS AND METHODS

4.1. Sample Preparation. Four different cultivars of olives (Arbosana, Arbequina, Chiquitita, and Koroneiki) were harvested during six time points in 2017: November 6, November 13, November 20, November 27, December 4, and December 11. Unfortunately, the reference method data on December 11 for Koroneiki are missing. The maturity indexes of the olive fruits were collected for each time points, as shown in Table 2.

Olives were crushed in a laboratory scale hammer mill (MC2 Ingenieria y sistemas S.L., MM-100, Spain) equipped with interchangeable grids (4, 6, and 8 mm) and a blade crusher (Mori-Tem S.R.L., FRANGITORE 150, Italy), as shown in Figure 1 within 24 h of harvesting.

4.2. Reference Moisture Determination. Olive samples were dried in a convection oven after being crushed with 4 mm grid size laboratory hammer mill. The percent moisture of the fruits was determined by gravimetry. The samples were placed in the oven at 105 °C for 10 h. Dried pastes were then removed from the oven and cooled in the desiccator prior to weighing.[28] The percent moisture content of the sample is given by eq 1.[8]

$$\%\ \text{moisture} = \left(\frac{M_{\text{wp}} - M_{\text{dp}}}{M_{\text{wp}}}\right) \times 100 \tag{1}$$

4.3. Reference Fat Determination. After olives were crushed with 4 mm grid size laboratory hammer mill and dried in a convention oven, the dried olive paste was manually crushed with a mortar to increase solvent-solid contact area. Soxhlet extractions were then performed using 20 g of dried paste. The paste was loaded in a cellulose extraction thimble, transferred to the Soxhlet apparatus, and extracted with *n*-hexane for 6 h. Once the extraction was finished, samples were placed in a rotary evaporator and oven at 105 °C for at least 3 h.[28] The dried paste fat content is calculated by eq 2 as shown.

$$\%\ \text{fat}_{\text{dp}} = \frac{(M_{\text{flask}} + M_{\text{oil}}) - M_{\text{flask}}}{M_{\text{dp}}} \times 100 \tag{2}$$

4.4. NIR Instrumentation and Spectral Acquisition. A portable NIR spectroscopy (Galaxy NIR QuasIR 3000) was used to measure the fat and moisture contents of wet paste olives. Triplicates of three different grid sizes (4, 6, 8 mm) from the hammer mill and the blade crusher were used as samples. The wet olive paste was contained in a quartz Petri dish with a pathlength of 1 mm such that it covered the Petri dish completely. The acquisition of spectra was obtained using a 90 mm diameter glass sample spinner that rotates at a constant speed to improve the NIR source light exposure to sample. The spectra were collected in the range of 4000−10 000 wavenumbers (cm^{-1}). Each spectrum consisted of 60 co-added scans with a resolution of 8 cm^{-1}. The scans were performed such that three different scans were averaged by the acquisition software to generate a single spectrum. Each of the 23 samples was partitioned into three parts to sample it three times by both near-infrared spectrometry and by traditional lab analysis.

Multivariate regression partial least squares (PLS) calibration model was formulated to characterize spectral variables with olive fat and moisture contents. The PLS calibration model was built using fruits crushed with 4 mm grid size on the hammer mill. For building the calibration, the average value of the lab result was used as the true value for all replicate scans of the sample. Separate models using QTA Chemometrics software were created for fat and moisture contents by utilizing approximately 50% of the data sets for calibration and the remaining 50% for model accuracy testing. The prediction residuals of the data set reserved for testing were computed as the difference between the lab value for the trait minus the PLS model prediction of the trait for each sample in the test set. The overall prediction error for the data set is referred to as the standard error of prediction (SEP) and computed as the root-mean-squared error of the residuals as follows

$$\text{SEP} = \sqrt{\frac{\sum (x_i - X_i)^2}{n}} \tag{3}$$

where x is a result computed from the PLS algorithm applied to sample i, X is the lab value based on wet chemistry for sample i, and n is the number of samples. The SEP is a measure of performance for a calibration model based on n tested values in the data set, which serves as the testing set. The calibration for both fat and moisture was optimized using the PLS software tool, which tests various wavelength regions, mathematical preprocessing functions, which are applied to the spectral data, as well as the number of regression coefficients used in the model. The models, which produce the lowest SEP values for the test set, are selected as the most effective models. For the fat algorithm, the preprocessing was single normal variate (SNV) standardization of the mean-centered spectra with the regions of 3996−7000 and 8199−9401 cm^{-1} used for the model. For moisture, SNV was also used, and the regions selected were 4595−5199 and 8199−10 000 cm^{-1}. Although the model was built with only samples from the 4 mm hammer mill, it was used to predict hammer mill grid sizes of 6, 8 mm and blade crushed olives to identify whether different crushing methods provide different results. SEP and SEC were also considered.

4.5. Statistical Analyses of Reference Method and NIR Spectra. Linear regression was performed on the average values of the triplicate reference method results and NIR prediction results to identify the relationship between both methods. Since dry basis results were obtained from the reference method, results were transformed to wet basis using the following eq 4.

$$\text{fat}_{\text{wp}} = M_{\text{dp}} \times \frac{(1 - M_{\text{moisture}})}{100} \quad (4)$$

ANOVA was used to determine the impact of different crushing conditions. Tukey multiple comparisons test was then performed to assess differences among means.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was funded by the Olive Oil Commission of California.

REFERENCES

(1) Dag, A.; Kerem, Z.; Yogev, N.; Zipori, I.; Lavee, S.; Ben-David, E. Influence of time of harvest and maturity index on olive oil yield and quality. *Sci. Hortic.* **2011**, *127*, 358−366.

(2) Ayora-Cañada, M.; Muik, B.; García-Mesa, J.; Ortega-Calderón, D.; Molina-Díaz, A. Fourier-transform near-infrared spectroscopy as a tool for olive fruit classification and quantitative analysis. *Spectrosc. Lett.* **2005**, *38*, 769−785.

(3) Barros, M.; Nunes, A.; Martins, J.; Delgadillo, I. Determination of oil and water in olive and olive pomace by NIR and multivariate analysis. *Sens. Instrum. Food Qual. Saf.* **2009**, *3*, 180−186.

(4) Cayuela, J.; Camino, M. Prediction of quality of intact olives by near infrared spectroscopy. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 1209−1217.

(5) Cayuela, J.; García, J.; Caliani, N.; et al. NIR prediction of fruit moisture, free acidity and oil content in intact olives. *Grasas Aceites* **2009**, *60*, 194−202.

(6) Dupuy, N.; Galtier, O.; Le Dréau, Y.; Pinatel, C.; Kister, J.; Artaud, J. Chemometric analysis of combined NIR and MIR spectra to characterize french olives. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 463−475.

(7) Hourant, P.; Baeten, V.; Morales, M.; Meurens, M.; Aparicio, R. Oil and fat classification by selected bands of near-infrared spectroscopy. *Appl. Spectrosc.* **2000**, *54*, 1168−1174.

(8) Muik, B.; Lendl, B.; Molina-Diaz, A.; Perez-Villarejo, L.; Ayora-Canada, M. Determination of oil and water content in olive pomace using near infrared and raman spectrometry. A Comparative Study. *Anal. Bioanal. Chem.* **2004**, *379*, 35−41.

(9) Saha, U.; Jackson, D. Analysis of moisture, oil, and fatty acid composition of olives by near-infrared spectroscopy: development and validation calibration models. *J. Sci. Food Agric.* **2018**, *98*, 1821−1831.

(10) Armenta, S.; Moros, J.; Garrigues, S.; Guardia, M. The use of near-infrared spectrometry in the olive oil industry. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 567−582.

(11) Bendini, A.; Cerretani, L.; Di Virgilio, F.; Belloni, P.; Lercker, G.; Toschi, T. In-process monitoring in industrial olive mill by means of FT-NIR. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 498−504.

(12) Morales-Sillero, A.; Fernández-Cabanás, V.; Casanova, L.; Jiménez, M.; Suárez, M.; Rallo, P. Feasibility of NIR spectroscopy for non-destructive characterization of table olive traits. *J. Food Eng.* **2011**, *107*, 99−106.

(13) Galtier, O.; Dupuy, N.; Le Dréau, Y.; Ollivier, D.; Pinatel, C.; Kister, J.; Artaud, J. Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. *Anal. Chim. Acta.* **2007**, *595*, 136−144.

(14) Marquez, A. J.; Diáz, A. M.; Reguera, M. I. P. Using optical NIR sensor for on-line virgin olive oils characterization. *Sens. Actuators, B* **2005**, *107*, 64−68.

(15) Mailer, R. J. Rapid evaluation of olive oil quality by NIR reflectance spectroscopy. *J. Am. Oil Chem. Soc.* **2004**, *81*, 823−827.

(16) Conte, L. S.; Brussolo, G.; Pizzale, L.; Carazzolo, A.; Meurens, M.; Pavan, O. Application of near infrared reflectance analysis to olive oil production quality control. *Riv. Ital. Sostanze Grasse* **2003**, *80*, 213−217.

(17) Mesa, J. A. G.; Fernández, M. H.; Alonso, P. C. Determination of oil and moisture in two-phases olive-pomace by using near-infrared spectroscopy. *Grasas Aceites* **1996**, *47*, 317−322.

(18) Salguero-Chaparro, L.; Baeten, V.; Abbas, O.; Peña-Rodríguez, F. On-line analysis of intact olive fruits by Vis−NIR spectroscopy: optimisation of the acquisition parameters. *J. Food Eng.* **2012**, *112*, 152−157.

(19) Szalay, A.; Antal, I.; Zsigmond, Z.; Marton, S.; Erős, I.; Regdon, G.; Pintye-Hódi, K. Study on the relationship between particle size and near infrared diffuse reflectance spectroscopic data. *Part. Part. Syst. Charact.* **2005**, *22*, 219−222.

(20) Jimenez, A.; Izquierdo, E.; Rodriguez, F.; Duenas, J. I.; Tortosa, C. Determination of fat and moisture in olives by near-infrared reflectance spectroscopy. *Grasas Aceites* **2000**, *51*, 311−315.

(21) Beltrán, G.; Rio, C. D.; Sanchez, S.; Martinez, L. Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. *J. Sci. Food Agric.* **2004**, *84*, 1783−1790.

(22) Lorenzo, L. Determination of oil and moisture content in intact olive fruits by portable near infrared spectroscopy. *NIR news* **2012**, *23*, 4−8.

(23) Sakouhi, F.; Herchi, W.; Sebei, K.; Absalon, C.; Kallel, H.; Boukhchina, S. Accumulation of total lipids, fatty acids and triacylglycerols in developing fruits of *Olea Europaea* L. *Sci. Hortic.* **2011**, *132*, 7−11.

(24) Büning-Pfaue, H. Analysis of water in food by near infrared spectroscopy. *Food Chem.* **2003**, *82*, 107−115.

(25) Otsuka, M. Comparative particle size determination of Phenacetin bulk powder by using Kubelka-Munk Theory and Principal Component Regression analysis based on near-infrared spectroscopy. *Powder Technol.* **2004**, *141*, 244−250.

(26) Inarejos-García, A. M.; Fregapane, G.; Salvador, M. Effect of crushing on olive paste and virgin olive oil minor components. *Eur. Food Res. Technol.* **2010**, *232*, 441−451.

(27) Guerrini, L.; Migliorini, M.; Giusti, M.; Parenti, A. The influence of crusher speed on extra virgin olive oil characteristics. *European J. Lipid Sci. Technol.* **2016**, *119*, No. 1600156.

(28) International Olive Council. Guide for the Determination of the Characteristics of Oil-Olives, 2001.