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Analytical Methods

Characterization and authentication of a novel vegetable source of omega-3 fatty acids, sacha inchi (*Plukenetia volubilis* L.) oil

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ABSTRACT

Consumption of omega-3 fatty acids (ω-3's), whether from fish oils, flax or supplements, can protect against cardiovascular disease. Finding plant-based sources of the essential ω-3's could provide a sustainable, renewable and inexpensive source of ω-3's, compared to fish oils. Our objective was to develop a rapid test to characterize and detect adulteration in sacha inchi oils, a Peruvian seed containing higher levels of ω-3's in comparison to other oleaginous seeds. A temperature-controlled ZnSe ATR mid-infrared benchtop and diamond ATR mid-infrared portable handheld spectrometers were used to characterize sacha inchi oil and evaluate its oxidative stability compared to commercial oils. A soft independent model of class analogy (SIMCA) and partial least squares regression (PLSR) analyzed the spectral data. Fatty acid profiles showed that sacha inchi oil (44% linolenic acid) had levels of PUFA similar to those of flax oils. PLSR showed good correlation coefficients ($R^2 > 0.9$) between reference tests and spectra from infrared devices, allowing for rapid determination of fatty acid composition and prediction of oxidative stability. Oils formed distinct clusters, allowing the evaluation of commercial sacha inchi oils from Peruvian markets and showed some prevalence of adulteration. Determining oil adulteration and quality parameters, by using the ATR-MIR portable handheld spectrometer, allowed for portability and ease-of-use, making it a great alternative to traditional testing methods.

1. Introduction

Native to the Peruvian jungles, sacha inchi (*Plukenetia volubilis* L.), also known as the "Inca peanut" or "wild peanut", grows at altitudes between 200 and 1500 m (Follegatti-Romero, Piantino, Grimaldi, & Cabral, 2009) and is a plant of the Euphorbiaceous family (Hamaker et al., 1992). The flour and oil from the seeds are commonly used by the Peruvian natives. The seeds contain approximately, on average, 48% oil and 27% proteins that are rich in cysteine, tyrosine, threonine and tryptophan (Guillén, Ruiz, Cabo, Chirinos, & Pascual, 2003). Sacha inchi was highest in oil content, between soybean and cottonseed, but comparable to sunflower and peanut (Hamaker et al., 1992). Sacha inchi seeds have a unique fatty acid composition containing a large amount of unsaturated fatty acids (about 85% polyunsaturation), comprised of approximately 34% linoleic acid (ω-6) and 51% linolenic acid (ω-3) (Guillén et al., 2003). These essential ω-6 and ω-3 fatty acids offer important health and nutritional benefits, such as providing protection against cardiovascular disease (Guillén et al., 2003), which is the number one killer disease in the United States (CDC, 2010). They also protect against rheumatoid arthritis, cancer and possibly the severity of viral infections (Fernandes & Venkatraman, 1993).

The primary sources of the essential polyunsaturated fatty acids (PUFA's) are fish oils, which are rich in these beneficial fatty acids (Venegas-Caleron, Sayanova, & Napier, 2010). Fish oil contains ω-6 (0.9–12 g/100 g oil) and ω-3 (11.9–35.3 g/100 g oil) (Rubio-Rodriguez et al., 2010) fatty acids. Production and processing of fish oils is a costly manufacturing process, aimed at removing colour pigments, contaminants (dioxins, furans and/or polyaromatic hydrocarbons) and volatile components responsible for the oil's odour and flavour (Bimbo, 2011). There is concern regarding the sustainability of fish, due to its decreasing populations from decades of over-fishing (Pauly, Watson, & Adler, 2005). Environmental pollution has resulted in the accumulation of dioxins and heavy metals in fish and must be tested to rule out dangerous levels of pollutants (Yokoo et al., 2003). Due to potential contamination, the benefits of obtaining PUFA's from fish are being questioned (Yokoo et al., 2003). It is cheaper and easier to remove oil from flaxseed, which is a renewable material, whereas fish is a diminishing source. Nevertheless, to produce docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from flaxseed oil, α-linolenic acid (ALA) needs be converted which is an inefficient process (Huang, Pereira, & Leonard, 2004).

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Due to the nutritional benefits of oils containing high levels of ω-3's, the potential for adulteration by unscrupulous manufacturers, for economic gain (by using low cost ingredients for the replacement, partially or totally, of high-cost ingredients), is a concern (Tay, Singh, Krishnan, & Gore, 2002). Adulteration could threaten the health of consumers, so detection of adulteration is a priority. Adulteration of oils is becoming more common due to its economic profits; therefore, a more sophisticated and rapid method of detecting adulteration is needed compared to the traditional chromatographic methods (Gurdeniz & Ozen, 2009). The development of a robust and reliable system, combined with multivariate analysis to monitor chemical processes occurring during lipid oxidation, to detect adulteration and determine fatty acid composition, is of great importance, in terms of both public health and consumer protection.

ATR-MIR benchtop and portable handheld spectrometers are relatively recent applications of spectroscopy when combined with chemometric analysis for determining authenticity of oils. Fourier-transform infrared (FT-IR) spectroscopy, equipped with a temperature-controlled ZnSe-ATR (attenuated total reflectance) accessory, is a great alternative for detecting adulteration compared to traditional methodologies, including chromatographic methods (Gurdeniz & Ozen, 2009).

The objective of this study was to develop a rapid test, combining ATR-MIR spectroscopy with chemometrics, to characterize and detect adulteration in sacha inchi oils. In addition, the oxidative stability performance of sacha inchi oils was compared with flax, high oleic sunflower and corn oils.

2. Materials and methods

2.1. Materials

Vegetable oils (3 of each; corn, canola, flax, cottonseed, sunflower, high oleic sunflower and olive) were purchased from a local grocery stores in Columbus, OH. A total of 17 sacha inchi samples were used in this study. Eight authentic sacha inchi samples used to create the models were supplied from UNALM (Peru). Commercial sacha inchi samples (numbered 1–9) purchased from local grocery stores (Lima, Peru) were used to test the models.

2.2. Reference methods

Peroxide value (PV) was determined using the AOCS official method Cd 8–53 (Firestone, 1998).

Free fatty acid (FFA) value was determined, using the AOCS official method Ca 5a-40 (AOCS (American Oil Chemists' Society), 1993).

Methyl ester fatty acid analysis was performed to determine the fatty acid composition of the oils, using a fatty acid methyl ester (FAME) procedure (Firestone, 1995; Rodriguez-Saona, Barrett, & Selivonchick, 1995). Esterification was achieved by adding 10 ml of 4% methanolic-sulfuric acid to 0.5 g oil samples plus 1 ml of benzene in a glass tube with a Teflon screw-top cap. Methyl esters were extracted using a partition of 2 ml of hexane and 1 ml of distilled water. 1 ml of the hexane portion was collected in a 1.5 ml vial and evaporated under nitrogen. The samples were re-diluted using 0.5 ml of iso-octane. Methyl esters were analyzed in a HP6890 GC equipped with a flame ionization detector (FID). An HP G1513A autosampler and tray were used to automate the injections. Separation of the components was done using an HP-FFAP 25 m × 0.32 mm × 0.5 μm column, using helium as the gas carrier. The injection volume was 1 μl with a split ratio of 20:1. The oven conditions were 110 °C (1 min), to 220 °C (5 °C/min), held for 15 min. The injector temperature was 220 °C and the detector temperature was 250 °C. Fatty acids were identified by comparing the retention times against reference standards (NuChek Prep GC standard 15A, Nu-Chek Prep, Inc., Elysian, MN).

Oil samples were analyzed individually, in duplicate, and statistical significance of the differences between mean values was assessed by one-way ANOVA with Minitab® 14.0 statistical software (Minitab Inc., State College PA). Tukey's multiple comparison procedure was used to compare the means for each reference test. A probability to $p \leqslant 0.05$ was used to establish the statistical significance.

2.3. Monitoring oxidative stability

To prepare the accelerated oxidative stability conditions, 50 ml of each oil (corn, high oleic sunflower, flax and sacha inchi) were measured into ten glass jars with lids (total of 40 jars, 2 for each oil each day). Uncovered jars were heated to 65 ± 1 °C in an incubator to accelerate oxidative conditions. The jars were left uncovered to expose the oils to the circulating air and to facilitate oxidation. Every 5 days for 20 days, a jar of each oil was removed from the incubator and stored at −40 °C prior to analysis. PV (AOCS official method Cd 8-53), FFA (AOCS official method Ca 5a-40) and methyl ester fatty acid analysis (Firestone, 1995; Rodriguez-Saona et al., 1995) reference methods were used, as well as ATR-MIR benchtop and portable handheld spectra collection with multivariate analysis (to analyze the data).

2.4. FT-IR spectroscopy

Infrared spectral data were collected on ATR-MIR benchtop and portable handheld spectrometers. Oil samples were heated in an oven to 65 °C prior to data collection for each spectrometer and measured in duplicate. The TruDefender™ FT infrared portable handheld spectrometer (Thermo Scientific), which was originally designed for field identification of unknown materials, is equipped with a diamond ATR crystal. The handheld unit had a spectral range of 4000–650 cm^{-1} and spectral data were collected by co-adding 4 scans at a resolution of 4 cm^{-1}. Infrared spectra were also collected on a benchtop Varian Excalibur 3100 spectrometer (Varian, Palo Alto, CA, USA) equipped with a KBr beam splitter and deuterated triglycine sulfate (DTGS) detector. A single-bounce FatIR™ temperature-controlled ATR ZnSe crystal (Harrick Scientific, Pleasantville, NY, USA) was set to 65 °C. Spectra were collected over a range of 4000–700 cm^{-1} at 4 cm^{-1} resolution and an interferogram of 64 scans, and co-added. Spectral data were displayed in terms of absorbance and viewed using Resolutions Pro Software. The instrument was purged continuously with CO_2 from a CO_2RP140 dryer (Dominic Hunter, Charlotte, NC, USA) to prevent interference in the spectra.

2.5. Data analysis

The spectra were analyzed using multivariate statistical analysis software, called Pirouette® (version 4.0, Infometrix Inc., Woodville, WA, USA). The spectra were imported into the software from the instruments as GRAMS (.spc) files and analyzed by normalizing, mean-centring and taking the second derivative for each spectra. Soft independent modelling of class analogy algorithm (SIMCA) was used to classify the oil samples, based on their type. SIMCA's discriminating power plot was used to identify which infrared bands were responsible for classification. Second derivative partial least squares regression (PLSR) models, with cross-validation, were used to study the correlation between the infrared spectrum of oil and PV, FFA values and fatty acid composition. Models were cross-validated, using a leave-one-out approach.

In SIMCA, the principal components contain information about influential chemical components that define the classes. By determining the F-statistic, an upper limit for residual variance (noise)

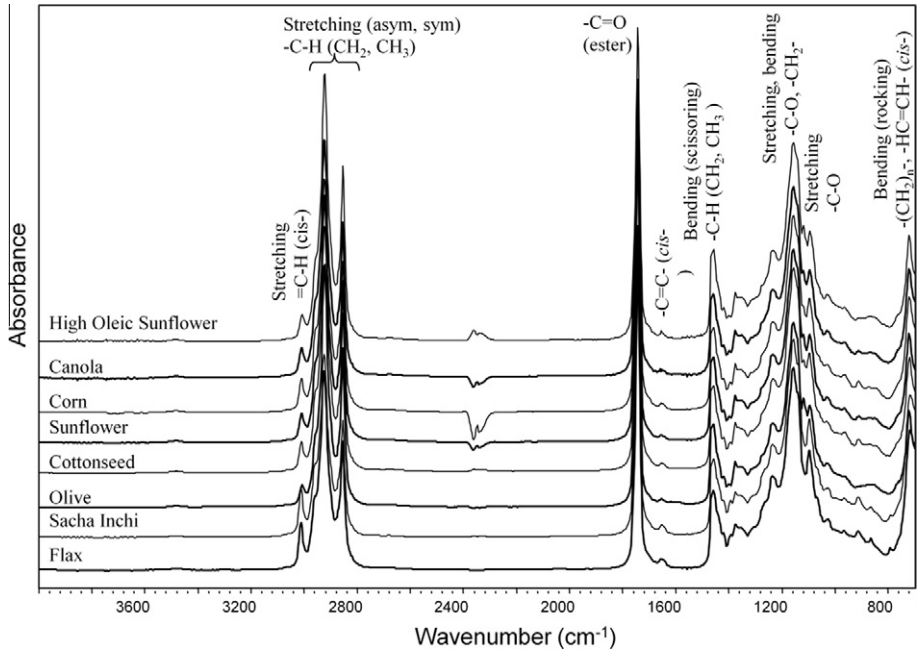

Fig. 1. FT-IR mid-infrared spectra of high oleic sunflower, canola, corn, sunflower, cottonseed, olive, sacha inchi and flax oils, using a temperature-controlled single-bounce ZnSe ATR mid-IR.

can be calculated for all samples belonging to each class, resulting in a set of probabilities of class-membership for each sample (boundary cloud). Thus, an unknown sample can only be assigned to the class for which it has a high probability. If the residual variance of a sample exceeds the upper limit for the modelled classes in the data set, it is not assigned to any of the classes; either it is an outlier, or it comes from a class not represented in the data set (Lavine, 2000). PLSR is a bi-linear regression, based on the extraction of latent variables (Bjorsvik & Martens, 1992). These orthogonal factors (latent variables) explain most of the covariance of the X (spectra) and Y variables. PLSR reduces the dimensions contained in thousands of IR predictors into a few factors to explain variations in both the dependent variables and the spectral domain. The ideal end-result is a linear model able to predict a desired characteristic (disease status), based on a selected set of predictors (IR spectra). PLSR has been particularly successful in developing multivariate calibration models for the spectroscopy field, because it reduces the impact of irrelevant X-variations (noise) in the calibration model (Bjorsvik & Martens, 1992; Martens & Naes, 1989). This capability provides a more information-rich data set of reduced dimensionality and eliminates data noise that results in more accurate and reproducible calibration models. The quality of the final model was evaluated, based on the number of latent variables, loading vectors, standard error of cross validation (SECV), coefficient of determination (R-value), and outlier diagnostics. Sample residual and Mahalanobis distance were used to determine outliers.

3. Results and discussion

3.1. Characterization and authentication of sacha inchi oils

The ATR-MIR spectrum of several commercial vegetable oils and sacha inchi (*Plukenetia volubilis* L.) (Fig. 1) oils provided unique

Table 1
Fatty acid composition for commercial vegetable oils (olive, canola, cottonseed, corn, high oleic sunflower, sunflower, flax and sacha inchi) and pure authentic sacha inchi oils using fatty acid methyl ester (FAME) procedure.

Oil sample	Fatty acid (%)[a]				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Olive	11.8 (±0.7)	2.8 (±0.06)	74.3 (±2.1)	8.4 (±1.0)	0.6 (±0.02)
Canola	5.2 (±0.09)	2.3 (±0.03)	64.1 (±0.3)	20.2 (±0.3)	6.6 (±0.2)
Cottonseed	22.4 (±0.9)	2.8 (±0.05)	18.4 (±0.5)	52.8 (±0.7)	0.5 (±0.03)
Corn	11.2 (±0.5)	2 (±0.09)	28.5 (±0.5)	56 (±0.2)	0.9 (±0.1)
High Oleic Sunflower	5.1 (±0.05)	2.7 (±0.05)	78.6 (±0.03)	11.6 (±0.06)	0.1 (±0.001)
Sunflower	4.7 (±0.05)	3.8 (±0.05)	60.2 (±0.03)	29 (±0.05)	0.37 (±0.0007)
Flax	5.6 (± 0.2)	4.4 (±0.05)	20 (±0.8)	15.5 (±0.5)	53.4 (±0.007)
Authentic Sacha Inchi	4.67 (±0.3)	3.5 (±0.1)	10.7 (±0.6)	33.5 (±1.0)	44 (±1.3)
Commercial Sacha Inchi Oils[b]	4.8 (±0.3)	3.4 (±0.1)	10.1 (±0.5)	37.7 (±1.6)	42.4 (±1.3)
Suspicious Sacha Inchi samples based on information from the infrared systems[c]					
2	12.6	3.8	18.8	53.5	7.1
4	4.5	3.2	11.0	37.2	42.3
9	9.7	4.1	19.6	49.6	14.5

[a] Relative % of fatty acid based on GC chromatographic peak area.
[b] Averages of sacha inchi samples (1, 3, & 4–8) that were verified as authentic by the temperature-controlled ZnSe ATR-IR and portable handheld diamond ATR-IR.
[c] Sacha inchi samples that were not clusterd as authentic by either the temperature-controlled ZnSe ATR-IR or portable handheld diamond ATR-IR.

information about different triglyceride substitution patterns due to degree and form of unsaturation of the acyl groups and their fatty acid chain length (Guillén & Cabo, 1997). Major spectral differences were evident in the 3050–2800 cm^{-1} region associated with the stretching vibration of the *cis* olefinic =C–H double bonds (3010 cm^{-1}) and the methylene asymmetrical and symmetrical stretching vibrations (2950 and 2845 cm^{-1}). In addition, flax and sacha inchi oils showed unique spectral bands in the 1120–1000 cm^{-1} range and 725 cm^{-1} which resulted from the stretching vibrations of the –C–O groups of esters derived from primary and secondary alcohols and the rocking vibrations of methylene group overlapping with bending vibration on out-of-plane mode of alkenes with *cis*-disubstituted olefinic groups, respectively (Guillén & Cabo, 1997). Flax and sacha inchi oils showed a more intense peak at 3010 cm^{-1} due to their higher degree of polyunsaturated fatty acids. Flax and sacha inchi oils contained approximately 68% (53% linolenic acid (ω-3) and 15.5% of linoleic acid (ω-6)) and 78% (44% linolenic acid and 33.5% of linoleic acid) polyunsaturated fatty acids (Table 1), respectively, which is in good agreement with levels reported by Guillén et al. (2003). By contrast, olive and high oleic sunflower oils showed the least intense bands at 3010 cm^{-1} due to their predominance of oleic acid (~76%).

FT-IR ATR, combined with a multivariate analysis, has been successfully implemented as a reliable, quick and simple technique for analyzing food products, edible oils and lipids (Birkel & Rodriguez-Saona, 2011). Soft independent modelling of class analogy (SIMCA) is a classification procedure based on principal component analysis (PCA) that groups samples into their classes, based on

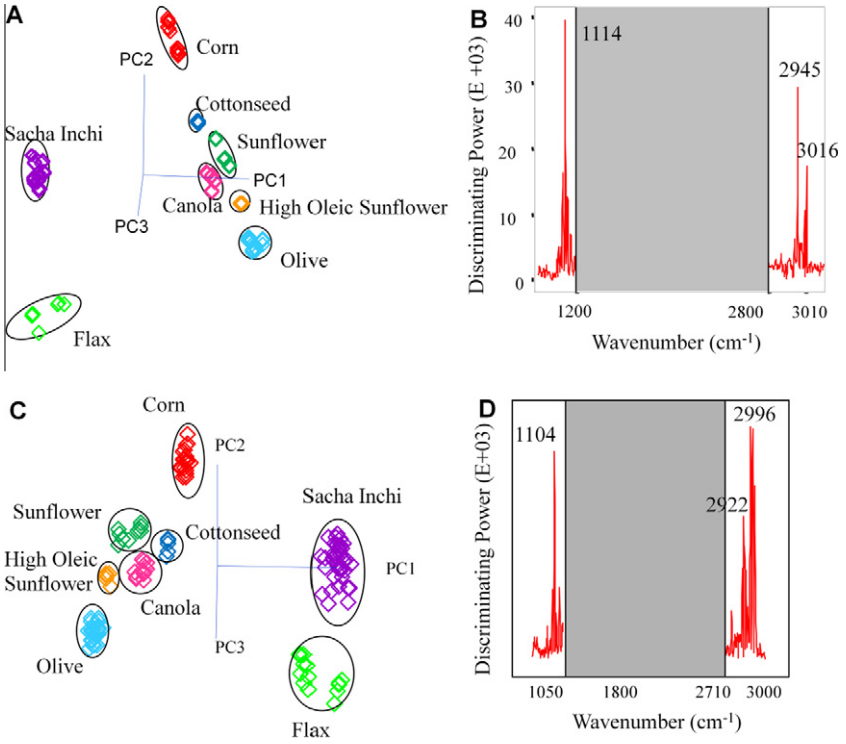

Fig. 2. Soft independent modelling of class analogy (SIMCA) 3D projection plots of second derivative-transformed spectral data collected by mid-IR ATR benchtop (A) and mid-IR ATR portable handheld (C) spectrometers. SIMCA discrimination plots for the mid-IR ATR benchtop (B) and mid-IR ATR portable handheld (D) spectrometers. For SIMCA projection plots; boundaries marked around the samples clustered represent a 95% confidence interval for each class. If the residual variance of a sample exceeds the upper limit of the boundary for the modelled classes in the data set, it is not assigned to any of the classes; either it is an outlier, or it comes from a class not represented in the data set.

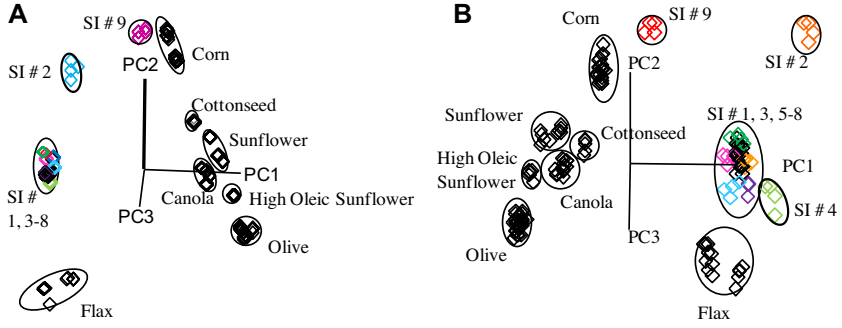

Fig. 3. SIMCA prediction plots for authentication of sacha inchi oils using the mid-IR ATR benchtop (A) and mid-IR ATR portable handheld (B) spectrometers. For SIMCA projection plots, boundaries marked around the sample clustered represent a 95% confidence interval for each class. If the residual variance of a sample exceeds the upper limit of the boundary for the modelled classes in the data set, it is not assigned to any of the classes; either it is an outlier, or it comes from a class not represented in the data set.

differences in their composition (infrared bands) (Allendorf, Subramanian, & Rodriguez-Saona, 2011). A class space is built whose boundaries discriminate between the samples fitting the class model and the samples that cannot be considered as belonging to the studied class.

Pattern recognition analysis by the SIMCA method, using the spectral data collected from a mid-infrared single-bounce ZnSe ATR-MIR benchtop (Fig. 2A) and a diamond ATR-MIR portable handheld system (Fig. 2C) showed well-separated clustering for the different oils.

SIMCA reduces the dimensionality in multivariate data sets so that systematic variation can be separated for each class and the residual variance (noise) is used to define the class boundaries (Vogt & Knutsen, 1985) around each class model. Thus, objects with residual standard deviation above the critical value (95% confidence interval) are considered as outliers or identified as samples not belonging to a group (Vogt & Knutsen, 1985).

Models generated with the ATR-MIR benchtop and ATR-MIR portable handheld spectrometers had interclass distances (ICD) ranging from 8.4 to 141 and 1.66 to 23.6, respectively, with flax and sacha inchi oils having the lowest ICD in both models. An ICD greater than 3 is used to determine if classes are significantly different among each other and the greater this ICD the greater the difference in their chemical composition (Allendorf et al., in

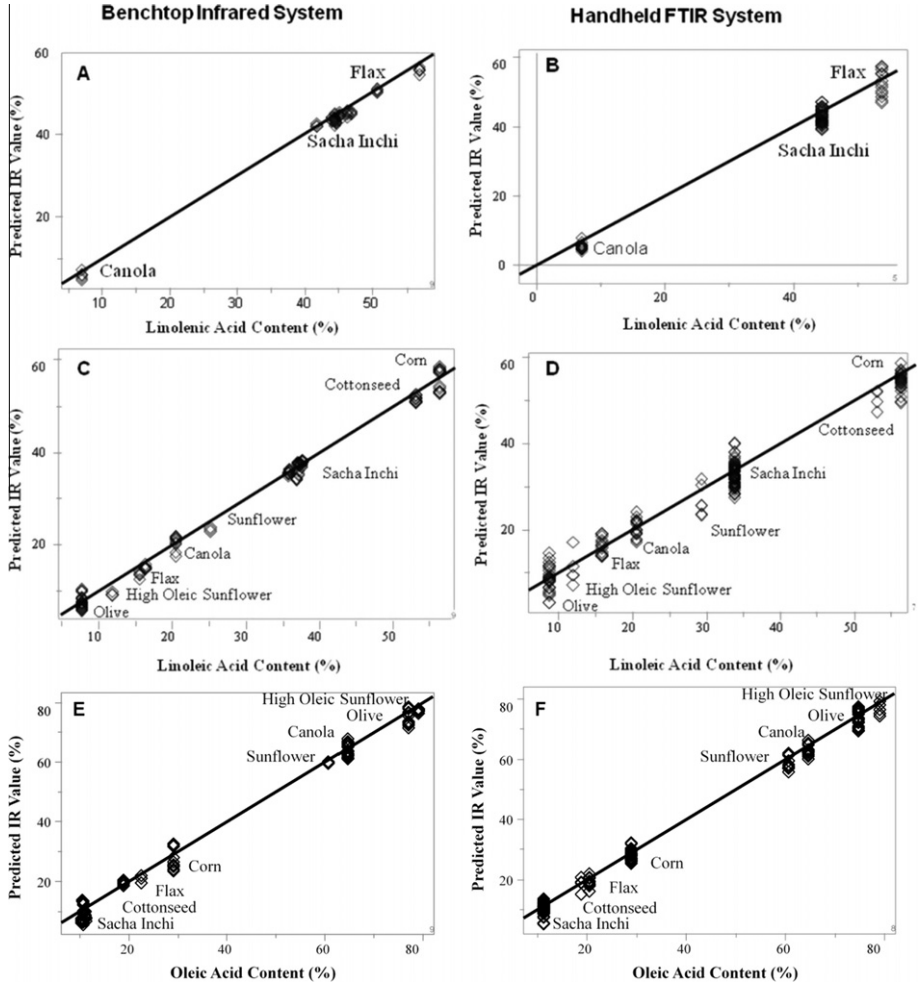

Fig. 4. Partial least squares regression (PLSR) of free fatty acids using the mid-IR ATR benchtop (A – linolenic acid, C – linoleic acid, E – oleic acid) and the mid-IR ATR portable handheld spectrometers (B – linolenic acid, D – linoleic acid, F– oleic acid).

Table 2
PLSR model statistical analysis for determining fatty acid composition in oils (corn, high oleic sunflower, flax and sacha inchi), using a benchtop and hendheld infrared system.

MIR technique		Concentration range (%)[a] (IR regions (cm1))[b]	Factors	SECV[c]	*r* Val.[d]
Temperature-controlled single-bounce ZnSe ATR-IR	Oleic	9.6–76.6 (940–1860; 2770–3100)	9	2.0	1.00
	Linoleic	7.4–56.0 (920–1860; 2780–3090)	8	1.07	1.00
	Linolenic	6.5–56.5 (950–1820; 2780–3100)	9	0.89	1.00
Portable handheld unit single-bounce diamond ATR-IR	Oleic	10.6–78.6 (940–1855; 2775–3080)	7	2.7	0.99
	Linoleic	8.4–56 (920–1870; 2770–3090)	7	2.65	0.99
	Linolenic	0.1–53.4 (950–1830; 2760–3100)	5	1.71	1.00

[a] Relative % of fatty acid based on GC chromatographic peak area.
[b] Selected frequencies (cm^{-1}) regions used to build the PLSR model.
[c] Standard Error of Cross Validation.
[d] Correlation of Cross Validation Model.

press). The SIMCA discriminating power plots explained the differences in functional groups responsible for the separation of classes. The higher the intensity of the band, the greater is its influence in discriminating the oil samples. Fig. 2B shows that most of the variance in the ATR-IR benchtop model was explained with bands at 1114 cm^{-1}, corresponding to asymmetric –C–O ester stretching vibrations. The ATR-MIR portable handheld (Fig. 2D) spectrum had a peak in the same region (1104 cm^{-1}), also corresponding to differences in esters derived from secondary alcohols present in the triglyceride molecule. In addition, both SIMCA models showed the influence of bands between 2922 and 3016 cm^{-1}, corresponding to $-CH_2$ asymmetric stretching, on differences in the fatty acid chain length between the oils and the *cis*- olefinic group =CH, assignable to the degree of polyunsaturated fatty acids, respectively.

The health and nutritional importance of the ω-3 polyunsaturated acyl groups provide protection against cardiovascular disease, rheumatoid arthritis and cancer (Fernandes & Venkatraman, 1993), creating consumer demands for foods supplemented with ω-3 acyl groups. Sales of omega-3 products are expected to reach $8.2 billion by 2012 (Packaged Facts., 2009) and 63% of consumers are trying to add omega-3 fatty acids to their diets. Consequently, adulteration of high-cost ingredients (ω-3 polyunsaturated rich oils) with lower grade, cheaper substitutes could potentially lead to economic fraud and serious health implications to consumers (Lai, Kemsley, & Wilson, 1994). Our SIMCA classification, using a set of pure sacha inchi oils, provided by the Universidad Nacional Agraria (Lima, Peru), was used to authenticate commercial sacha inchi oils purchased from local markets in Lima (Peru). The SIMCA plots generated using the spectra collected from the ATR-MIR benchtop (Fig. 2A) and portable handheld (Fig. 2C) spectrometers were used to predict authenticity of 9 commercial sacha inchi oil samples. Predictions made from the ATR-MIR benchtop (Fig. 3A) and portable handheld (Fig. 3B) SIMCA models showed that samples #2 and #9 were clustered away from the pure sacha inchi oil class. Data from the GC methyl ester fatty acid composition reference method showed that samples #2 and #9 had higher linoleic (50–54%) and lower linolenic (7–14.5%) acid as compared to pure sacha inchi samples (Table 1). Therefore, the data revealed that samples #2 and #9 are not authentic and likely have been adulterated with another vegetable oil. Interestingly, the same manufacturer made these two oils. The ATR-MIR portable handheld SIMCA prediction plot also showed that sample #4 was outside of the typical sacha inchi class boundary. The fatty acid composition reference method showed that this sample had levels of polyunsaturated fatty acids similar to those of the pure sacha inchi samples. This false positive prediction (non-authentic) by the ATR-MIR portable handheld spectrometer could be attributed to measurement uncertainty due to environmental or sampling factors, leading to increased noise and therefore, the false positive.

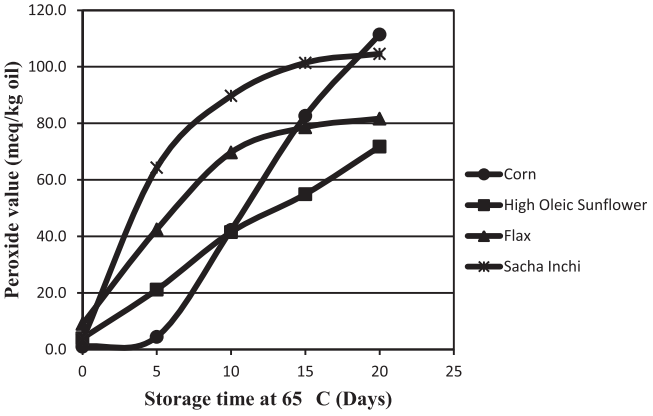

Fig. 5. Peroxide values (PV) of corn, high oleic sunflower, flax and sacha inchi oils versus the storage time (in days) in a 65 °C oven.

Partial least squares regression (PLRS) models were created by combining the infrared spectral data with the quantitative measurements obtained from the GC methyl esters fatty acid composition, peroxide value (PV) and free fatty acid (FFA) reference methods. The power of the PLS method is based on its ability to mathematically correlate spectral changes with changes in the concentration of a component of interest (van de Voort, Ismail,

Table 3
Peroxide value (PV), free fatty acid (FFA), and fatty acid composition for the different oil samples studied during a 20 day oxidative stability test.

Oil	Reference test	Storage (days)				
		0	5	10	15	20
Corn	PV (meq/kg oil)	1 (±0.001)	4.5 (±0.4)	42.3 (±2.8)	82.6 (±1.4)	111.4 (±0.1)
	FFA (%)	0.3 (±0.001)	0.5 (±0.01)	0.65 (±0.02)	0.67 (±0.04)	0.7 (±0.01)
	Saturated fat (g/100 g oil)[a]	13.7 (±0.4)	13.8 (±0.1)	14.2 (±0.04)	14.3 (±0.2)	14.3 (±0.6)
	Monounsaturated fat (g/100 g oil)[b]	30.5 (±0.5)	30.4 (±0.3)	30.8 (±0.2)	31.2 (±0.09)	31.2 (±0.5)
	Polyunsaturated fat (g/100 g oil)[c]	54.7 (±0.1)	55.4 (±0.3)	54.8 (±0.1)	54.4 (±0.2)	54.5 (±0.7)
High oleic sunflower	PV (meq/kg oil)	4 (±0.1)	21.2 (±1.1)	41.5 (±1.0)	54.9 (±1.4)	71.7 (±2.1)
	FFA (%)	0.38 (±0.001)	0.45 (±0.01)	0.5 (±0.01)	0.54 (±0.001)	0.52 (±0.001)
	Saturated fat (g/100 g oil)[a]	6.5 (±0.001)	6.5 (±0.07)	6.6 (±0.01)	6.5 (±0.05)	6.4 (±0.04)
	Monounsaturated fat (g/100 g oil)[b]	77.9 (±0.02)	78.2 (±0.2)	78.3 (±1.5)	78.3 (±0.5)	78.1 (±0.2)
	Polyunsaturated fat (g/100 g oil)[c]	15.4 (±0.02)	15.1 (±0.07)	13.6 (±1.4)	14.7 (±0.1)	14.8 (±0.1)
Flax	PV (meq/kg oil)	9.06 (±0.01)	42.5 (±2.1)	69.7 (±2.1)	78.6 (±3.5)	81.6 (±0.001)
	FFA (%)	0.65 (±0.01)	0.85 (±0.01)	0.94 (±0.03)	0.95 (±0.01)	0.95 (±0.001)
	Saturated fat (g/100 g oil)[a]	9.4 (±0.3)	9.9 (±0.1)	10.1 (±0.05)	10.2 (±0.001)	6 (±0.001)
	Monounsaturated fat (g/100 g oil)[b]	22.1 (±0.8)	24.4 (±0.6)	25.1 (±0.7)	26.2 (±0.1)	23.9 (±0.2)
	Polyunsaturated fat (g/100 g oil)[c]	64.8 (±0.4)	64.1 (±0.2)	63.4 (±0.1)	63.3 (±0.2)	62.9 (±0.3)
Sacha Inchi	PV (meq/kg oil)	3.4 (±0.01)	64.3 (±1.5)	89.7 (±2.2)	101.4 (±2.3)	104.6 (±1.4)
	FFA (%)	0.36 (±0.02)	0.58 (±0.03)	0.7 (±0.01)	0.7 (±0.03)	0.75 (±0.001)
	Saturated fat (g/100 g oil)[a]	7.4 (±0.2)	7.9 (±0.02)	8.2 (±0.08)	8.1 (±0.001)	8.3 (±0.05)
	Monounsaturated fat (g/100 g oil)[b]	10 (±0.4)	10.1 (±1.3)	10.6 (±0.09)	11 (±0.9)	12.5 (±0.1)
	Polyunsaturated fat (g/100 g oil)[c]	80.9 (±0.4)	80.2 (±0.09)	78.9 (±0.09)	80 (±1.6)	78.3 (±0.8)

All concentrations were determined using the GC-FAME method, as reported in Table 1.
[a] Saturated fat refers to relative percent GC chromotographic peak area for palmitic plus stearic fatty acids.
[b] Monounsaturated refers to relative percent GC chromatographic peak area for oleic acid.
[c] Polyunsaturated refers to the relative percent GC chromatographic peak area for linoleic plus linolenic fatty acids.

Sedman, Dubois, & Nicodemo, 1994). PLS regression plots generated for linolenic (ω-3) (Fig. 4A & 4B), linoleic (ω-6) (Fig. 4C & 4D) and oleic (ω-9) (Fig. 4E & 4F) acids showed good correlation between the fatty acid levels and the estimated concentrations using the ATR-MIR systems. Table 2 shows that the regressions developed from using the spectral data obtained from the ATR-MIR benchtop had a lower SECV (0.9–2.0%) in predicting%fatty acid composition than had the ATR-MIR portable handheld (SECV 1.7–2.7). The leave-one-out cross validation method was used for selection of the optimal number of factors in the PLSR models, ranging from 5–9, depending on the infrared system used to collect the spectrum. Overall, the benchtop system used more latent variables (factors) due to its higher signal-to-noise ratio, extracting more statistically significant variation contributions associated with combinations of different chemical components.

3.2. Monitoring oxidative stability

The high content of unsaturated fatty acids makes the sacha inchi oils very susceptible to peroxidation under mild environmental conditions (Follegatti-Romero et al., 2009). Exposure of oils (corn, high oleic sunflower, flax and sacha inchi) to 65 °C for 20 days resulted in a marked increase in peroxide value (Table 3). Composition differences in the oils played a significant (p-value < 0.05) role in oxidation rates. Sunflower oil showed the highest oxidative stability while corn had high levels of peroxide formation at the end of 20 days (Table 3). Interestingly, corn oil showed the least oxidation in the first five days compared to the other oils, as shown in Fig. 5. This may be attributed to the presence of natural antioxidants, α-tocopherols, in corn oil that had been reported to inhibit the formation of hydroperoxides, but, once the tocopherols were depleted, the oxidation process in corn oil accelerated rapidly (Huang, Frankel, & German, 1994). The rate of peroxide formation in high oleic sunflower and corn (after an induction period) oils increased linearly over the storage time, as the rate of peroxide formation equals its decomposition during propagation reactions in the thermal lipid oxidation process. Sacha inchi and flax oils showed exponential trends in their peroxide values reaching a plateau after 15 days (Fig. 5) as peroxide decomposition exceeds the rate of formation, resulting in decreasing peroxide values (Orlein, Risbo, Rantanen, & Skibsted, 2006). Overall, sacha inchi and flax oils were the most oxidatively unstable, due to their large amounts of polyunsaturated fatty acids making them more susceptible to oxidation. Of the four oils compared in this study, high oleic sunflower oil had the largest amount of saturated and monounsaturated fatty acids, making it the most stable. The level of FFA found in the oils and rates of formation also varied according to the type of oil. In commercial practices, the oil is usually discarded after the FFA level reaches 1–1.5%, as there will be sufficient breakdown material present to rapidly catalyze further oxidation (Tseng, Moreira, & Sun, 1996). FFA values did not significantly change after the first 5 days; thus, using FFA values alone for monitoring oxidation, was not sufficient. Overall, there were no significant (p-value > 0.05) changes in saturated, monosaturated and polyunsaturated fatty acids during the accelerated oxidative study, as measured by the fatty acid methyl ester analysis, except for polyunsaturated levels in flax oil (p-value 0.015).

After 20 days of stressing the oils at 65 °C, SIMCA projection plots showed spectral differences within oil samples clustering according to storage day (0–20) (Fig. 6). The discriminating power plots showed changes at bands at 1705–1766 cm^{-1} (depending on the oil type) due to their thermal oxidation, which correlated with the carbonyl-stretching band from the acyl-linked hydrocarbon chains (Borchman & Sinha, 2002). All of the oils followed similar patterns in the SIMCA projection plots for spectra collected using

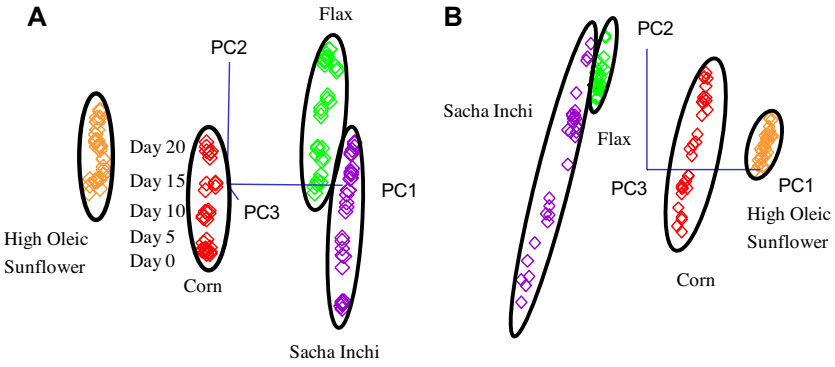

Fig. 6. Soft independent modelling of class analogy (SIMCA) 3D projection plots of second derivative-transformed spectral data collected by mid-IR ATR benchtop (A) and mid-IR ATR portable handheld (B) spectrometers for corn, high oleic sunflower, flax and sacha inchi oils during their oxidation process. For SIMCA projection plots, boundaries marked around the sample clustered represent a 95% confidence interval for each class. If the residual variance of a sample exceeds the upper limit of the boundary for the modelled classes in the data set, it is not assigned to any of the classes; either it is an outlier, or it comes from a class not represented in the data set.

Table 4
Statistical analyses for the PLSR models developed to determine the PV and FFA of oils (corn, high oleic sunflower, flax and sacha inchi) during their oxidation process using mid-IR ATR benchtop and mid-IR ATR portable handheld spectrometers.

MIR Technique		Concentration range (%)[a] (IR regions (cm^{-1}))[b]	Factors	SECV[c]	r Val.[d]
Temperature-controlled single-bounce ZnSe ATR- IR	PV (meq/kg oil)	1–66 (820–1850; 2745–3080)	8	2.06	1.0
		1–112 (825–1855; 2740–3075)	9	4.13	0.99
	FFA (%)	0.028–0.96 (965–1800; 2805–3070)	8	0.06	0.94
Portable handheld unit single-bounce diamond ATR-IR	PV (meq/kg oil)	1–66 (850–1880; 2730–3100)	6	4.97	0.97
		1–112 (860–1885; 2730–3100)	6	10.20	0.96
	FFA (%)	0.028–0.96 (960–1800; 2790–3100)	7	0.13	0.94

[a] Relative % of fatty acid based on GC chromatographic peak area.
[b] Selected wavelength (λ) regions used to build the PLSR model.
[c] Standard Error of Cross Validation.
[d] Correlation of Cross Validation Model.

the ATR-MIR benchtop (Fig. 6A) or portable handheld (Fig. 6B) instruments, with the former technique yielding greater interclass distances.

PLSR was used to correlate oxidative reference tests with spectral data from the ATR-MIR benchtop and portable handheld spectrometers. Table 4 gives the PLSR model statistical analysis for determining PV and FFA values for each of the four oils used in the oxidative stability test (corn, high oleic sunflower, flax and sacha inchi). The regressions developed from the spectral data obtained from the ATR-MIR benchtop had a lower SECV in predicting both the PV and FFA value than had the ATR-MIR portable handheld spectrometer. Rancidity becomes noticeable in oils with peroxide values above 20 meq/kg, as noted by Gulla, Waghray, and Reddy (2010); therefore the PV is reported in two concentration ranges, providing lower SECV values, using the lower range. In this study, most oils were greater than 20 meq/kg by day 5, with the exception of corn oil. Therefore our study supports combining PV and FFA for monitoring oxidative stability.

4. Conclusions

A temperature-controlled ZnSe ATR-MIR benchtop and diamond ATR-MIR portable handheld spectrometers have proven to be fast, reliable and rapid techniques to characterize and authenticate sacha inchi oil. The ATR-MIR portable handheld instrument gave spectral information comparable to the ATR-MIR benchtop but there was a slight increase in noise. Sacha inchi oil (44%) has a ω-3 composition similar to that of flax (53%), but it had approximately twice as much ω-6 fatty acids. Combining FT-IR spectroscopy with chemometrics has proven to be a great alternative to traditional testing methods. SIMCA projection plots formed distinct clusters for each oil class, making prediction about oil fatty acid composition easy. PLSR showed good correlation coefficients ($R^2 > 0.9$) between reference tests and spectra from infrared devices, allowing for rapid determination of fatty acid composition and predicting oxidative stability.

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