Rapid Evaluation of Olive Oil Quality by NIR Reflectance Spectroscopy

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ABSTRACT: NIR spectroscopy calibrations have been developed for a range of quality parameters in olive oil, including FFA, PV, polyphenol content, induction time, chlorophyll, and the major FA. A set of 216 olive oil samples from throughout the Australian olive-growing areas were used to provide a representative range of quality. The variation in the oils tested virtually covered the range of the chemical standard limits described by the International Olive Oil Council. A FOSS NIRSystems® 6500 spectrophotometer with a liquid cell holder was used. Multiple correlation coefficients squared (R^2) for minor components stearic acid (0.86), and linolenic acid (0.85) were relatively low because the concentration range is very narrow compared with the reproducibility of the reference method. However, the major FA, oleic (0.99) and linoleic (1.00), FFA (0.97), and chlorophyll (0.98) provided high levels of accuracy. All of the parameters measured were sufficiently accurate for routine screening of olive oil.

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Olive oil consumption in Australia has risen dramatically in recent years, with imports of 29,000 tonnes in 2002. Simultaneously, domestic production has increased, with in excess of 7.5 million trees planted in Australia in the last 10 yr. The introduction of olives into Australia since the 1800s has been well documented (1), with the varieties generally having reference to European types although several recently imported varieties help to make up the gene pool. Recent studies in Australia (2) indicate considerable variation within and between named cultivars. Olive oil quality also varies considerably with environmental conditions, particularly in relation to water availability (3). Genetic variation together with Australia's varied environment contributes to a wide range in oil quality, both chemically and organoleptically.

The Australian industry has adopted the standards of the International Olive Oil Council (IOOC) (4) for the analysis and classification of olive oil. These standards classify oil into the categories of extra virgin, virgin, lampante, refined, and pomace oils. The current aim of processors in Australia is to produce only extra virgin oils and there are currently no refining facilities or solvent extraction plants for olive oils. The major quality components to be considered are FFA and PV, which are generally related to management practices. Additionally, the FA profiles, polyphenol content, and chlorophyll levels, which are related to both environmental and cultivar effects, contribute to the sensory and stability qualities of the oil. The analysis of all of these components requires considerable time and skill, together with a well-equipped laboratory. The opportunity to use the fast, simple, and nondestructive method of NIR spectroscopy has therefore been investigated. Such technology already has been shown to be useful for determining FA composition in seed of *Brassica napus* L. (5) and oxidative stability of vegetable oils (6). This study has used 216 oil samples from throughout the environmentally variable olive-growing areas of Australia. Chemical factors including FFA, PV, induction time, polyphenol content, and FA profiles have been calibrated for NIR analysis. The results provide evidence of the ability to measure most components rapidly and accurately.

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MATERIALS AND METHODS

Samples. Olive oil samples were obtained from growers throughout the Australian olive-growing regions. Samples were received in commercial bottles as prepared for the retail markets. The oils were analyzed over the period 16 April–15 August 2002. Two hundred sixteen samples were received although not all samples were tested for each of the chemical parameters.

Chemical analyses. All samples were obtained within 2 mon of pressing and transferred to 50-mL glass ampoules. Nitrogen gas was used to displace air in the space above the oil, and butyl seals were clamped onto the bottles. The samples were stored in an air-conditioned laboratory (20–25°C) in boxes away from light. The chemical composition and the range of the samples analyzed are presented in Table 1.

(i) FFA. FFA were determined by a modified method of AOCS Aa 6-38 (7); the modification involved replacement of 0.25 N sodium hydroxide (NaOH) with 0.1 N NaOH to increase the volume of titration and thereby increase the accuracy. Fresh 0.1 N NaOH was prepared daily.

(ii) PV. PV was determined using the IUPAC (8) method 2-501, as determined by the IOOC.

(iii) Total polyphenols (PP). PP were determined by a modification of the Gutfinger (9) method, as described previously (10), using caffeic acid as the standard. The standards were prepared and analyzed in the same way as the sample solutions.

(iv) Induction time. Induction time was measured using a Metrohm 679 Rancimat. A block temperature of 130°C was used with air flow of 20 L air/h.

TABLE 1
Minimum, Maximum, Mean, and SD for the Chemical Components of a Selection of Olive Oils[a]

	FFA (%)	PV (meq/kg)	PP (mg/kg)	Induction time (h)	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Chlorophyll
Minimum	0.06	2.6	40.2	1.33	10.61	0.68	1.28	56.54	3.86	0.57	0.00
Maximum	8.00	18.0	851.9	18.55	16.37	2.75	4.37	79.50	21.92	1.18	14.45
Mean	0.40	9.5	202.9	4.58	14.01	1.28	2.21	67.83	12.81	0.82	2.84
SD	0.59	3.6	147.6	2.81	1.33	0.33	0.58	4.20	3.54	0.13	2.85
Total no. of values	216	131	206	192	172	172	172	172	172	172	174
IOOC limits	<0.8%	<20 meq/kg	NA	NA	7.5–20.0	0.3–3.5	0.5–5.0	55.0–83.0	3.5–21.0	<1.0%	NA

[a]PP, polyphenol content; NA, not applicable; IOOC, International Olive Oil Council. Units for palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids are percent; units for chlorophyll are g/kg.

(v) Chlorophyll content. Chlorophyll content was determined using AOCS method Ch 4-91 (7).

FA profiles (FAP). FAP were determined as FAME, as previously described (10), by GC. Separation of the FAME was performed on a Varian 3800 gas chromatograph using a Supelco® BPX 70 (Supelco, Bellefonte, PA) chromatography column (30 m × 0.22 mm, 0.25 μm film) and FID. The column temperature was programmed at 185°C for 8 min, then increased at 10°C per min to 220°C. It was held for 3 min before cooling. The injector (split mode) temperature was set at 240°C with a split ratio of 1:50. The detector temperature was 250°C. Data were analyzed using Star Workstation Chromatography software (Version 6.2; Varian, Sydney, Australia).

NIR spectrophotometer. A Foss NIRSystems 6500 Near Infra Red Spectrophotometer fitted with a liquid cell holder, Model SY-1610-II, was provided by Foss Pacific (Sydney, Australia) for this study. The system was fitted with a digital sample temperature controller to provide constant sample temperature. The instrument was operated in transmission mode, providing data collection between 400 and 2500 nm. Spectral data over 2250 nm were not usable owing to the high absorbance from borosilicate disposable vials.

NIR calibration. WinISI II software (Foss Pacific) was used with a modified partial least squares calibration model, after applying a scatter correction SNV (standard normal variate) and detrend and first derivative math treatment (Fig. 1). Some outliers were deleted as follows: If any samples produced global outliers, indicating a spectrum totally different from all other spectra in the sample set, that sample was removed unless that outlier was caused by a sample similar to samples one might expect in the future. Samples that gave *t*-statistic outliers indicated values much higher or lower than the average concentration and SD of the overall predicted values. *t*-Statistic outliers were also deleted unless samples with this concentration were expected in the future. Residual outliers indicated large residual values between predicted and reference values. If the reference value was found to be wrong, the samples were deleted.

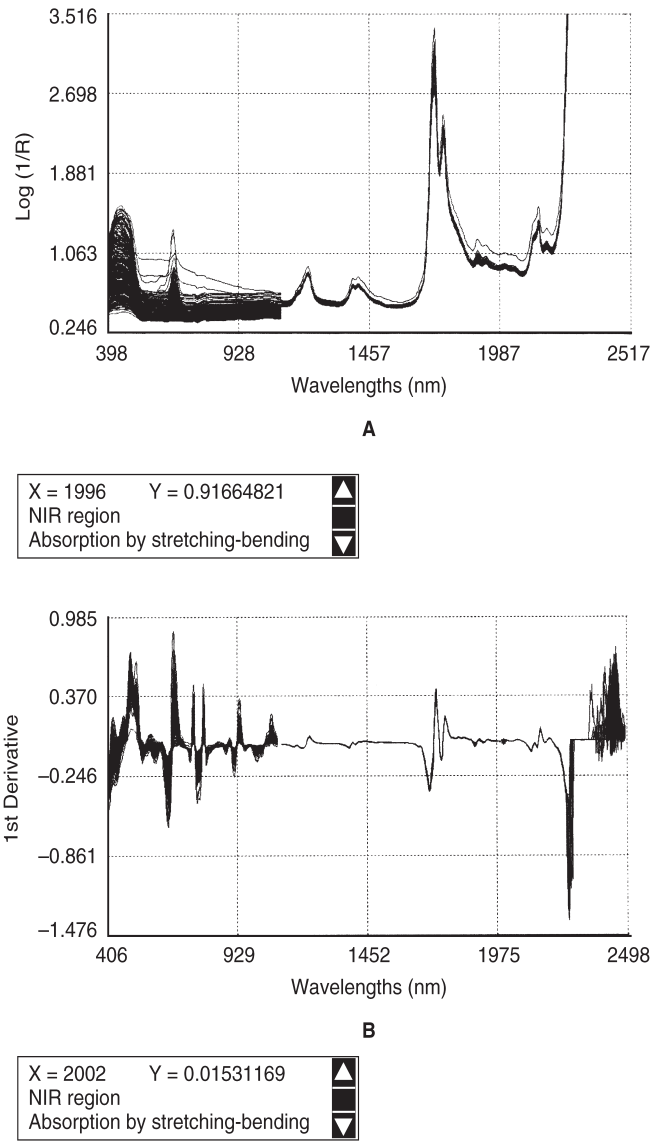

FIG. 1. NIR spectra of all samples included in the project (A) and the same spectra after scatter correction standard normal variate and detrend and first derivative (B).

RESULTS AND DISCUSSION

Chemical analysis. The extensive range of the oils used for this study is shown in Table 1. Not all of the parameters were tested on all of the 216 oils obtained. However, the range is indicative of the quality produced in Australian olive oils and is therefore adequate for NIR calibration. The permissible IOOC ranges for FFA, PV, and the individual FA are also shown in Table 1.

The spectra obtained from a scan of the samples are shown in Figure 1A (log 1/*R*) and Figure 1B (first derivative). With the initial calibrations and using the full spectral range from 400 to 2500 nm, the spectra in the region above 2250 nm appeared highly saturated, and there was considerable variation in the color region below 700 nm. The calibration was therefore repeated with a limited spectral range from 400 to 2250 nm and the correlation improved for polyphenol, stearic acid, and linolenic acid; however, chlorophyll decreased slightly. Statistical values for all parameters are shown in Table 2.

FFA contents of the oils were generally less than 0.8%, the IOOC maximum, although one sample was very high, at 8.0%. This sample was removed from the calibration as it was well outside the normal range of results. The initial calibration for FFA was carried out using 216 samples (Fig. 2A) and produced eight outliers, which were removed. Calibration over the full

TABLE 2
Statistical Values for 13 Components from Olive Oil Determined by NIR[a]

Constituent	*N*	Mean	SEC	R^2	1-VR	SECV
FFA	208	0.36	0.05	0.97	0.9445	0.07
PV	125	9.40	1.02	0.92	0.8546	1.34
PP	197	196.9	47.66	0.89	0.8233	58.67
Induction time	183	4.38	0.84	0.88	0.8339	0.97
Acids						
Palmitic	165	14.02	0.41	0.91	0.8639	0.48
Palmitoleic	166	1.26	0.11	0.87	0.8235	0.12
Stearic	158	2.16	0.20	0.86	0.7304	0.26
Oleic	167	67.77	0.38	0.99	0.9876	0.47
Linoleic	168	12.85	0.17	1.0	0.9958	0.23
Linolenic	162	0.82	0.05	0.85	0.7985	0.06
Chlorophyll	168	2.88	0.42	0.98	0.9685	0.51

[a]SEC, standard error of concentration on the calibration model; 1-VR, correlation coefficient of the cross-validation set; SECV, standard error of cross validation; R^2, multiple correlation coefficient; for other abbreviations see Table 1.

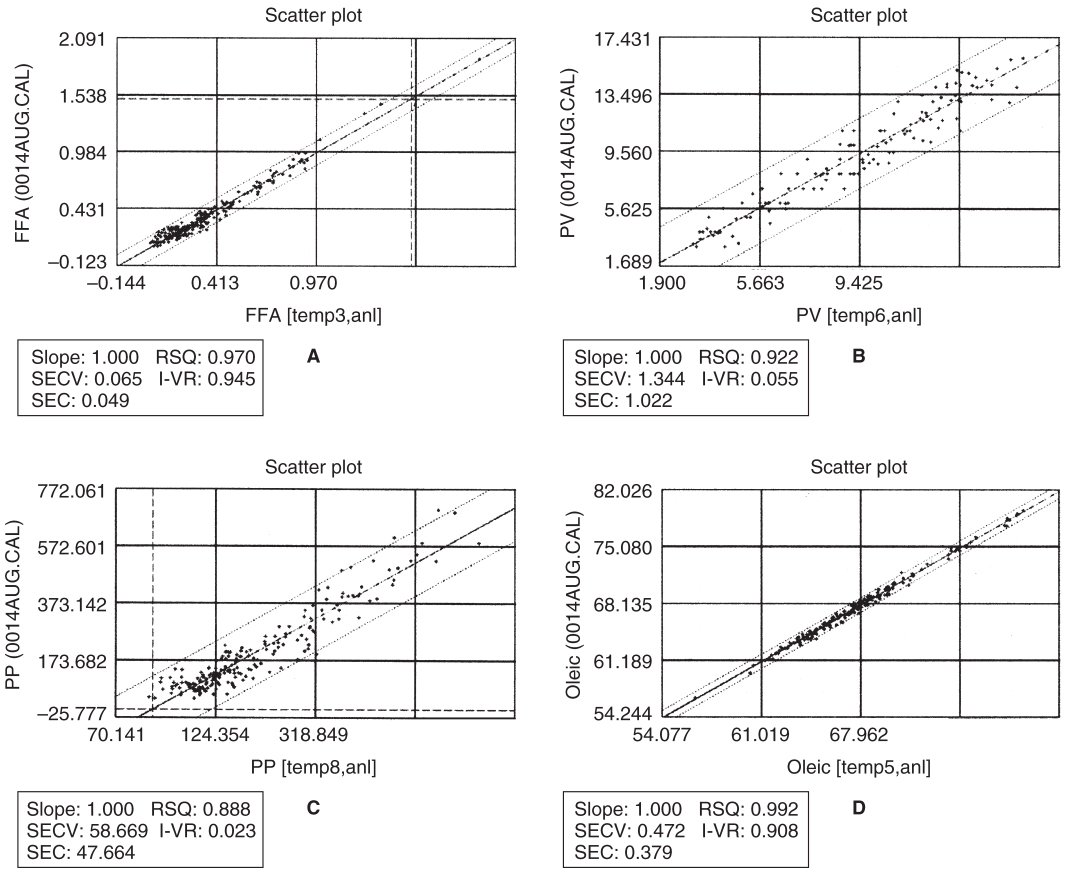

FIG. 2. Scatter plot diagram of chemical analysis against NIR prediction showing the correlations for each parameter measured: FFA (A); PV (B); polyphenols (PP) (C); oleic acid (D). SEC, standard error of concentration on the calibration model; 1-VR, correlation coefficient of the cross-validation set; SECV, standard error of cross validation.

wavelength range produced a 1-VR (correlation coefficient of the cross-validation set) of 0.838. This was improved with a reduced wavelength from 400 to 2200 nm to 0.945. A SEC (standard error of concentration on the calibration model) of 0.049 was achieved, as was a SECV (standard error of cross validation) of 0.065, both of which are adequate to screen olive oil samples. There was a relatively poor distribution of the samples over the range and a potential to improve the calibration with more data.

PV. PV virtually covered the IOOC range of <20 meq/kg for that component (2.6 to 18 g/kg). PV was calibrated on 131 samples, and six outliers were identified and removed (Fig. 2B). The 1-VR value was 0.855. The SEC of 1.022 was achieved, and the SECV was 1.344, which was also accurate enough to test oil samples with a standard range from 0 to 20 meq/kg. The samples were well distributed across the range, as illustrated by the histogram (Fig. 3A).

PP. There are no official limits for polyphenol content, but these samples, from 40 to 852 g/kg, provided sufficient variability for a calibration set. The polyphenol calibration was based on 197 samples after the removal of nine outliers (Fig. 2C) and had a 1-VR of 0.823. An SEC of 47.664 was achieved and an SECV of 58.669. Figure 2C indicates that samples were generally low in polyphenols and that a better calibration could be achieved with a wider distribution.

Induction time. Induction time also has no official standard but in this set ranged from 1.3 to 18.6 h, which provided an adequate range for the majority of samples analyzed through this laboratory. Induction time calibrations were based on 183 samples after the removal of nine outliers. The 1-VR value was 0.834. An SECV of 0.973 and SEC of 0.839 were achieved. Samples generally had low induction times, and the data set were not well distributed.

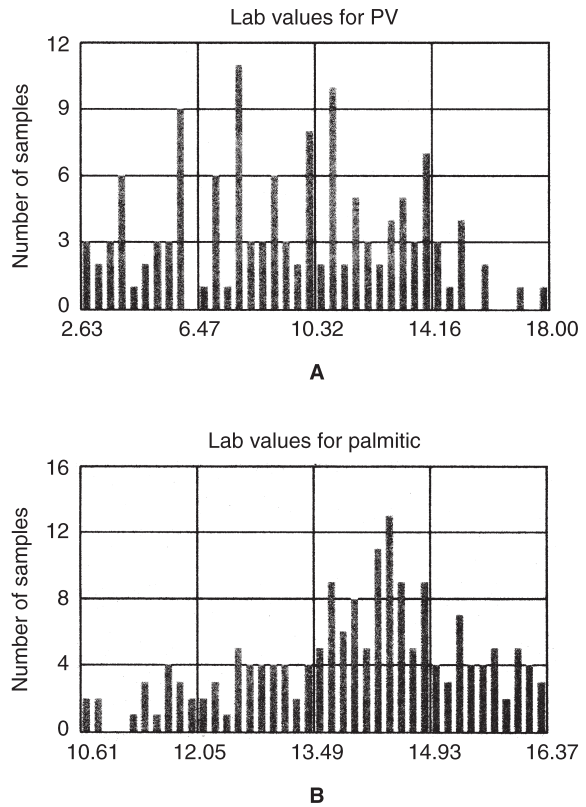

FIG. 3. Histograms of the distribution of samples over the range for two of the parameters measured: PV (A); palmitic acid (B).

FAP. Calibrations were obtained for all of the major FA with varying levels of success. The linoleic acid 1-VR value of 0.996 was excellent and produced an SECV of 0.231. Linoleic acid also had a good sample distribution, which may account for the result. Oleic acid (Fig. 2D) calibrations were also very good, with an 1-VR value of 0.988 and SECV of 0.472. There was also a good distribution for oleic acid across the range. Palmitic acid had an 1-VR of 0.864, also with samples evenly distributed across the range (Fig. 3B). Other FA had progressively weaker correlations although most were adequate for routine analysis. Palmitoleic and linolenic acids had 1-VR values of 0.82 and 0.80, respectively, whereas stearic acid was 0.73.

There is a need for accurate analysis of FA in olive oil as they need to fit within strict limits set by the IOOC. Several studies have illustrated variation in FA profiles under varying environmental conditions. Based on IOOC standards, olive oil that does not meet these limits cannot be referred to as olive oil but as fruit oil.

The set of samples collected from Australian growers represented a wide range of oils and oil quality. The parameters measured are those that are commonly tested in olive oil to indicate to the grower they have met standards required for extra virgin olive oil. The oil produced in Australia is all designed to be extra virgin olive oil, and currently there are no facilities in Australia for producing lower grades, such as olive pomace oil.

The error determined in this work has illustrated that some tests are less accurate than others. The relative performance of NIR analysis for each of the individual parameters is illustrated in Table 3. In the case of the polyphenols, the polyphenol chemical test leads to a low NIR prediction value. The induction time error also was almost 1 h (range 0–11.5), and this may also be expected in light of the methodology. Induction time has a strong relationship with polyphenol content, as the antioxidant effect of polyphenols assists in reducing oxidation (11). However, the error may be acceptable in many situations where ranges of polyphenols may be from 50 to 1000 g/kg.

TABLE 3
IOOC Limits and Relative NIR Performance for Each of the Quality Parameters[a]

Constituent	IOOC limits	Regression SECV	Estimate
FFA	0.8%	0.07	Good
PV	20 meq/kg	1.34	Good
Palmitic acid	7.5–20%	0.48	Good
Palmitoleic acid	0.3–3.5%	0.12	Poor
Stearic acid	0.5–5.0%	0.26	OK
Oleic acid	55–83%	0.47	Good
Linoleic acid	3.5–21.0%	0.23	Good
Linolenic acid	<1.0%	0.06	Inadequate
Polyphenol	NA (40–852)	58	Marginal
Induction time	NA (1.3–18.6)	0.97	OK
Chlorophyll	NA (0–14.5)	0.51	Good

[a]NA, no standard available. For other abbreviations see Tables 1 and 2.

Calibrations were excellent for the major FA including palmitic, oleic, linoleic, and linolenic acids. They were less acceptable for palmitoleic and stearic acids, where concentrations are naturally low and the variability in the samples is low. Chlorophyll also produced good correlations and low SECV values. However, in some cases, the sample numbers were insufficient to produce reasonable data sets with sufficient variation to achieve good correlations. This may be improved by increasing the number of samples and including a wider range of data.

Overall, the use of NIR has been shown here to have ample accuracy to be applied to daily, routine analysis. Where the need to meet international standards is required, the results could be used to indicate those samples that are clearly within range or those that are close to borderline or over the limit. This reduced set of samples could then be analyzed in more detail to obtain precise measurements and be classified accordingly. To implement this system, a larger database would need to be built up to further validate and improve its accuracy.

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