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Variation in Caffeine Concentration in Single Coffee Beans

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ABSTRACT: Twenty-eight coffee samples from around the world were tested for caffeine levels to develop near-infrared reflectance spectroscopy (NIRS) calibrations for whole and ground coffee. Twenty-five individual beans from five of those coffees were used to develop a NIRS calibration for caffeine concentration in single beans. An international standard high-performance liquid chromatography method was used to analyze for caffeine content. Coffee is a legal stimulant and possesses a number of heath properties. However, there is variation in the level of caffeine in brewed coffee and other caffeinated beverages. Being able to sort beans on the basis of caffeine concentration will improve quality control in the level of caffeine in those beverages. The range in caffeine concentration was from 0.01 mg/g (decaffeinated coffee) to 19.9 mg/g (Italian coffee). The majority of coffees were around 10.0−12.0 mg/g. The NIRS results showed r^2 values for bulk unground and ground coffees were >0.90 with standard errors <2 mg/g. For the single-bean calibration the r^2 values were between 0.85 and 0.93 with standard errors of cross validation of 0.8−1.6 mg/g depending upon calibration. The results showed it was possible to develop NIRS calibrations to estimate the caffeine concentration of individual coffee beans. One application of this calibration could be sorting beans on caffeine concentration to provide greater quality control for high-end markets. Furthermore, bean sorting may open new markets for novel coffee products.

KEYWORDS: *caffeine, coffee bean, ground coffee, bean sorting, near infrared spectroscopy*

INTRODUCTION

Coffee is one of the most-consumed beverages in the world with aroma and taste being the main sensory characteristics. The dominant commercial species of coffee are Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* Pierre), and most commercial coffee beverages are produced from these roasted beans or blends of these two. However, there are differences in quality and sensory profiles between these species, and suitable analytical methods and trained taste panels are required to quantify these differences.

In regard to quantifying chemical components, such as lipids, protein, or caffeine concentration, expensive and time-consuming wet chemistry methods are required. However, near-infrared reflectance spectroscopy (NIRS) has been used as a high-throughput and cheap surrogate for quantification of quality (including caffeine concentration)[1−7] and sensory parameters.[8] The differences detected in the chemical composition within and between Arabica and Robusta varieties can be useful for quality classification purposes, and NIRS has been used successfully in this context.[1] In addition, NIRS has also been used for quantification of green bean quality[9,10] and to understand genetic, environmental, and processing effects on quality.[11]

In most biological studies, a bulk sample is tested to obtain a representative mean value, although there is little information on the variation within the sample and the impact of the variation on the result. Several studies in cereal crops investigated the evaluation of single grains to ascertain the variation within a sample for a range of attributes using NIRS.[12−24] To date, there has been no report of assessment of single coffee beans by NIRS. However, single coffee beans have been tested for specific purposes including optimizing a wet chemistry method for caffeine concentration[25] and identifying breeding lines for reducing caffeine concentration.[2,26]

The aim of our study was to develop NIRS calibrations for caffeine concentration in bulk unground and ground coffees as well as single coffee beans using a high-performance liquid chromatography (HPLC) method as the reference method to quantify caffeine levels. The NIRS calibration in bulk unground and ground coffees would provide a rapid, predictive method of caffeine concentration. A NIRS calibration on single beans would provide the opportunity to understand the potential variability in coffee samples, for example, blends, or sort beans on the basis of caffeine concentration to further quantify quality and potentially develop new products (e.g., naturally low-caffeine coffee).

MATERIALS AND METHODS

Twenty-eight roasted Arabica and blended whole bean coffees, purchased from retail outlets, from a number of countries were used. Most of the coffees were Arabica type as per the information on the packet. Unfortunately, some of packets lacked specific or complete information on the origin or blend of the coffee. Table 1 shows the types and origins of coffees.

Ground Coffee. A 20 g subsample from each coffee was ground in a standard electric coffee grinder for NIR scanning and HPLC analysis. The grinder was cleaned with a fine brush between coffees.

FT-NIR. Three forms of coffees (bulk unground, ground, and single beans) were scanned in a Fourier transform (FT) NIR multipurpose analyzer instrument (Bruker, Germany) from 4000 to 12000 at 8 cm resolution. For bulk unground and ground coffees, the 97 mm cup on

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Table 1. Caffeine Concentration of Samples Collected

coffee	brand[a]	type[a]	origin[a]	caffeine (mg/g)
1	Brasilia Coffee Italian Espresso	blend	Italy	17.7
2	Brasilia Coffee Supa Cream	blend	Italy	12.7
3	Oxfam Aus World Blend Organic Espresso	Arabica	Africa/Latin American/Asia Pacific	12.5
4	Vittoria Espresso Coffee	blend	Italy	12.2
5	Caffe Aurora Medaglia Doro	blend	Italy	19.9
6	Monjava	blend	Central America	12.4
7	Dormans Breakfast (MR[b])	blend	Kenya	11.7
8	Dormans Espresso (DR[c])	blend	Kenya	11.8
9	Dormans AA Blue mountain (MR)	Arabica	Kenya	11.4
10	Masai (MR)	Arabica	Kenya	10.8
11	CTM Lush	blend	Colombia/Nicarigua/Timor/New Guinea	10.7
12	CTM House	blend	Brazil/Colombia/Sumatra/India	12.3
13	CTM Revive	blend	Colombia/Costa Rica/Indonesia/New Guinea/Brazil	11.6
14	CTM Jamocha	blend	South America/Central America/Africa/East India	12.7
15	CTM Mimic (decaf)	blend	Colombia/New Guinea	0.01
16	CTM Single Origin	blend	Rwanda	10.6
17	Woolworths Select Espresso	Arabica		12.2
18	Gloria Jean's Coffee Smooth Classic Blend	Arabica	Central and South America	12.1
19	North Queensland Gold	Arabica	Australia	11.6
20	PNG Hand Roast	Arabica	Papua New Guinea	12.2
21	Brazil Hand Roast	Arabica	Brazil	12.8
22	Colombia Hand Roast	Arabica	Colombia	12.8
23	Doisaket Coffee Brand	Arabica	Thailand	11.7
24	Native Organic	Arabica	Brazil	10.5
25	Green Cauldron	Arabica	Australia	10.7
26	Café One Italian No. 5	Arabica	Italy	11.8
27	Spar Espresso (SRR[d])	Arabica	South Africa	11.8
28	Café de Chamarel L'lle Maurice		Europe	11.7

[a]As per information on sample packet. [b]MR, medium roast. [c]DR, dark roast. [d]SR, strong rich roast.

the rotating sphere was used, where 64 scans of the rotating cup were averaged into a single spectrum. Coffees were scanned in duplicate.

Five coffees were selected for single-bean analysis (coffees 5, 7, 11, 15, and 24 in Table 1). Four samples were blends that would provide a broad range within each coffee. A single Arabica was also included. Twenty-five individual beans were randomly selected from each of these five coffees and scanned in a 19 mm glass vial using the microsphere attachment. A chrome insert was placed on top of each bean to prevent light escaping, as per Bruker recommendations. The wavenumber range and resolution were the same as for the bulk unground and ground coffees. After scanning, each bean was sealed in a plastic bag and stored in the freezer prior to grinding for caffeine analysis.

Opus (V 7.0) was used for calibration development with all pretreatments selected in the Optimise process to find optimal calibration math treatment and factors. Savistky–Golay, with first and second derviatives, was used with 4, 4 gap, and smoothing. Individual spectra files were exported in data point table format (Opus software), loaded into Excel, and then imported into Unscrambler [V 9.8, (Camo, Sweden)] for calibration development. The calibrations developed from these two multivariate software programs were compared using the Fearn[27] calculation, which tests for significant differences ($P < 0.05$) between the standard deviation of prediction errors of predicted values (from cross-validation) using both packages.

Caffeine Analysis. For the ground coffees, 2.000 g of coffee was weighed out on a three decimal place analytical balance. For single beans, each bean was ground in the coffee grinder and recovered. The recovered coffee was weighed and used for caffeine extraction.

The weighed coffee was dissolved in boiling water, mixed well, and kept heated for 10 min with constant stirring. The coffee solution was quickly cooled in iced water tand hen equilibrated at room temperature. The coffee solution was made up to 100 mL in a volumetric flask, mixed well, and then filtered through a no. 42 filter paper. Five milliliters of the filtrate was further filtered through a 0.45 μm mesh nylon membrane and diluted 1 in 100 mL in a volumetric flask for HPLC analysis.

A Waters HPLC system was used for caffeine quantification. Caffeine (see Figure 1 for structure) was separated in an Alltech Prevail C18, 150 mm × 4.6 mm, 5 μm, column with a guard column. Column temperature was 30 °C and injection volume, 10 μL. The mobile phase was 40% methanol and 0.5% phosphoric acid. The final pressure in the instrument was 119–122 bar with the detector set at 254 nm. Caffeine was identified by matching peak retention time against a caffeine standard and quantified by plotting against the standard curve of prepared caffeine standards at 1, 5, 10, and 20 mg/mL.

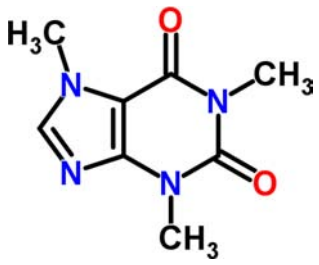

Figure 1. Structure of caffeine. Extracted from http://www.chemspider.com/Chemical-Structure.2424.html.

RESULTS

Range in Caffeine between Coffees. Of the 28 coffees collected in this study, one (no. 15) was a decaffeinated coffee, whereas all others were caffeinated. The caffeine concentration of the decaffeinated coffee was 0.01 mg/g (Table 1). The range in caffeine concentration for the other 27 samples was from 10.6 and 19.9 mg/g (Table 1). Most coffees were between 10.6 and 12.8 mg/g, whereas coffees 1 and 5 were much higher (17.7 and 19.9 mg/g, respectively). These two high-caffeine coffees were obtained from Italy and, as per the information on the packet, were identified as blends.

Range in Caffeine within Selected Coffees for Single-Bean Analysis. Five of the 28 samples were selected to investigate variation in caffeine concentration within each sample. Table 2 shows the HPLC caffeine concentrations for the 25 beans from the 5 coffees. For all coffees used in the single-bean analysis, the calculated average of the 25 individual beans was slightly less than the result for the coffee when tested as a bulk ground coffee (Table 2). However, all five of these coffees had individual kernels well above the bulk ground average. For the decaffeinated coffee, all beans contained trace amounts of caffeine, whereas the high-caffeine coffee had eight individual beans above 20 mg/g, with one kernel at 25.3 mg/g.

Table 2. Variation in Caffeine Concentration (Milligrams per Gram) in 25 Single Beans from 5 Selected Coffees

	sample				
bean	5	7	11	15	24
1	10.1	11.2	9.9	1.2	10.4
2	16.8	10.1	11.3	0.7	7.4
3	16.5	9.3	8.4	0.7	9.5
4	15.8	13.6	8.9	0.9	10.1
5	21.7	13.2	10.0	0.7	12.0
6	14.7	14.1	13.0	0.6	11.8
7	22.7	12.4	10.3	1.0	8.6
8	17.9	13.9	9.0	0.5	10.1
9	19.4	17	11.0	1.2	8.3
10	20.2	10.1	10.1	1.2	10.1
11	18.9	8.6	8.9	0.6	9.7
12	15.9	10.7	7.7	0.6	9.7
13	20.2	12.3	9.9	0.6	9.4
14	18.2	10.8	8.5	0.3	9.5
15	23.7	11.2	10.5	0.5	11.0
16	20.1	14.4	8.3	0.8	10.5
17	15.4	12	11.2	0.9	12.2
18	25.3	11	10.9	0.6	9.3
19	18	11.4	10.5	0.9	9.0
20	20.1	10.6	9.6	0.8	11.0
21	16.1	10	7.9	1.1	11.0
22	15.3	10	9.6	0.6	11.0
23	16.7	11.8	8.9	0.6	9.8
24	15.4	6.7	7.9	0.6	7.2
25	18.2	10.5	9.1	0.6	11.2
calcd mean	18.1	11.5	9.7	0.8	10.0
median	18.0	11.2	9.6	0.7	10.1
SD	3.2	2.8	1.2	0.3	1.3
max	25.3	14.4	13.0	1.2	12.2
min	14.7	6.7	7.7	0.6	7.2
ground coffee[a]	19.9	11.7	10.7	0.0	10.5

[a]Caffeine concentration of bulk ground coffee for each of these coffees.

The distribution plots of caffeine concentration of each of the individual five coffees is shown in Figure 2.

Figure 3 shows the distribution plots for the weight of beans used for calibration development. A normal distribution was seen in bean weights for caffeinated and decaffeinated beans. Panels a and b of Figure 4 show the correlation plots between caffeinated beans and decaffeinated beans, respectively, when compared to bean weights. There was a positive relationship in caffeine between caffeinated beans and bean weights (R^2 = 0.31). There was a similar positive relationship between bean weight and caffeine concentration in decaffeinated beans (R^2 = 0.34). These relationships indicated around 30% of bean weight is associated with caffeine concentration, which would suggest around 70% of the caffeine concentration is not explained by weight. Hence, selecting for weight would not assist in calibration development if the goal was to select for heavier or lighter beans.

NIR Calibrations for Bulk Unground and Ground Coffees. For all calibrations developed, either a standard normal variation (SNV) first derivative or multiplicative scatter correction (MSC) first derivative was selected as best. All calibrations for bulk unground and ground beans had greater than eight factors. The Opus calibration for bulk unground and ground coffees resulted in high coefficients of determination of

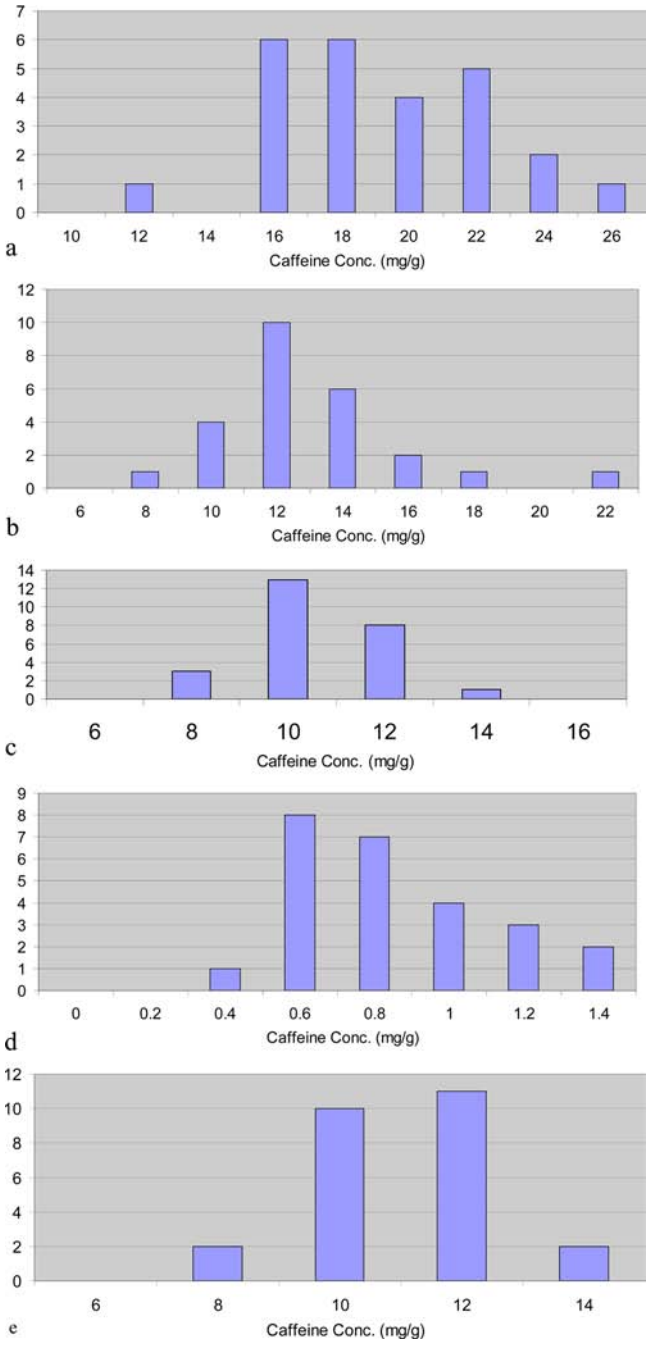

Figure 2. Distribution of caffeine between single beans of five samples: 5 (a); 7 (b); 11 (c); 15 (d); 24 (e).

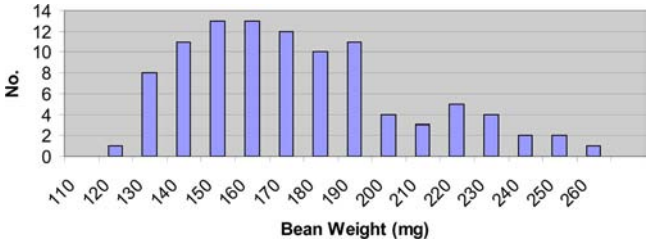

Figure 3. Distribution of bean weights used in NIRS calibration development.

0.93 and 0.95, respectively (Table 3), whereas in Unscrambler the r^2 values were 0.92 and 0.95, respectively. The r^2 values for

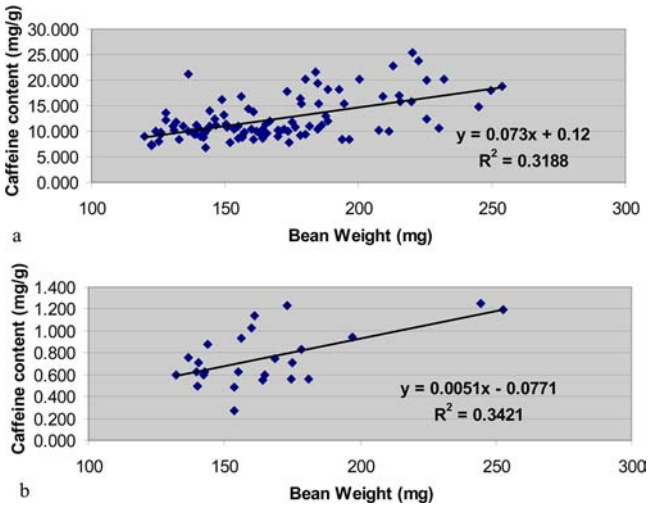

Figure 4. Correlation plot between bean weight and caffeine concentration for use in NIR calibration development for (a) caffeinated coffees and (b) decaffeinated coffee beans.

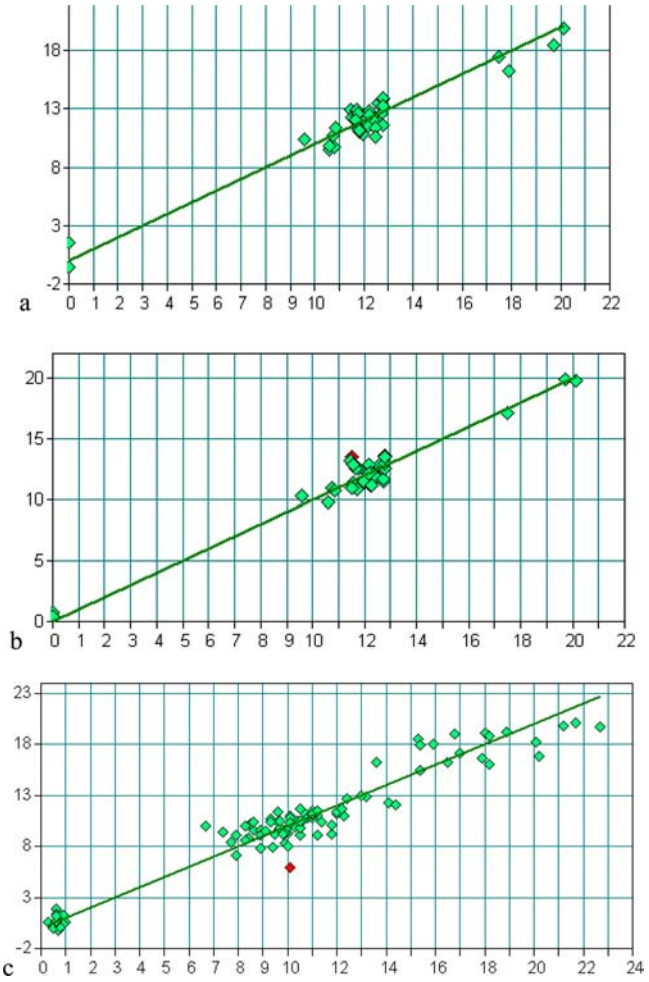

Figure 5. Correlation plots for whole (a), ground (b), and (c) single-bean coffees [actual (*X* axis) versus predicted (*Y* axis)].

internal cross-validation were ≥0.92 for bulk unground or ground coffees. The NIRS correlation plots for the whole bulk unground and ground coffees are shown in Figures 4a and 5b, respectively.

The inclusion of a single decaffeinated coffee (coffee 15), with a zero level of caffeine well below the next coffee at 10.5 mg/g could have a major influence on the overall regression. Hence, the calibrations were repeated with the decaffeinated coffee excluded. The cross-validation r^2 values were again >0.90 for unground and ground coffees using both multivariate analysis programs.

All standard error of calibration (SEC) results for whole bulk unground and ground coffees were <1.2 and <1.3 mg/g, respectively, by both modeling packages (Table 3). The SECs for the calibrations excluding the decaffeinated coffee showed a slight improvement.

Both multivariate analysis packages allowed for internal cross-validation. The internal standard error of cross-validation (SECV) for both unground and ground coffee was <1.3 mg/g (Table 3). The SECV decreased slightly when the decaffeinated coffee was excluded from the calibration set.

A ratio of standard error prediction to standard deviation (RPD) is used to indicate the potential application value of the

Table 3. Statistics for NIR Calibrations for Unground Bulk, Ground, and Single-Bean Coffees[a]

sample type	no.	R^2	SEC	R^2_{CV}	SECV	RPD_{CV}	factors	bias	treatment (smoothing)
Opus calibrations									
bulk	28	0.9325	0.9	0.9255	1.0	3.65	8	0.003	2dSNV (9)
ground	28	0.9504	0.8	0.9333	0.9	4.10	5	0.001	2dSNV (9)
single bean	124	0.9510	1.4	0.9197	1.7	3.53	10	0.005	1dSNV (13)
bulk less decaff coffee	27	0.9311	0.9	0.9178	1.0	3.55	8	0.003	2dSNV (9)
ground less decaff coffee	27	0.9434	0.8	0.9199	0.9	4.25	5	0.001	2dSNV (9)
single-bean less decaff coffee	99	0.9304	1.1	0.8749	1.5	2.83	9	0.02	1dMSC (13)
Unscrambler calibrations									
bulk	28	0.9225	0.9	0.9255	1.0	3.65	8	0.003	2dSNV
ground	28	0.9511	0.8	0.9343	0.9	4.10	5	0.001	2dSNV
single bean	122	0.9491	1.3	0.9228	1.6	3.55	8	−0.013	1dSNV
bulk less decaff coffee	27	0.9301	0.9	0.9098	1.0	3.52	8	0.003	2dSNV
ground less decaff coffee	27	0.9407	0.8	0.9124	0.9	4.21	5	0.001	2dSNV
single-bean less decaff coffee	97	0.9275	1.2	0.8599	1.6	2.75	8	−0.02	1dSNV

[a]No., number of samples used in the calibration; R^2, coefficient of determination of calibration set; SEC, standard error of calibration; R^2_{CV}, coefficient of determination of cross-validation; SECV, standard error of cross-validation; RPD_{CV}, ratio of SD to standard error of prediction using the cross-validation.

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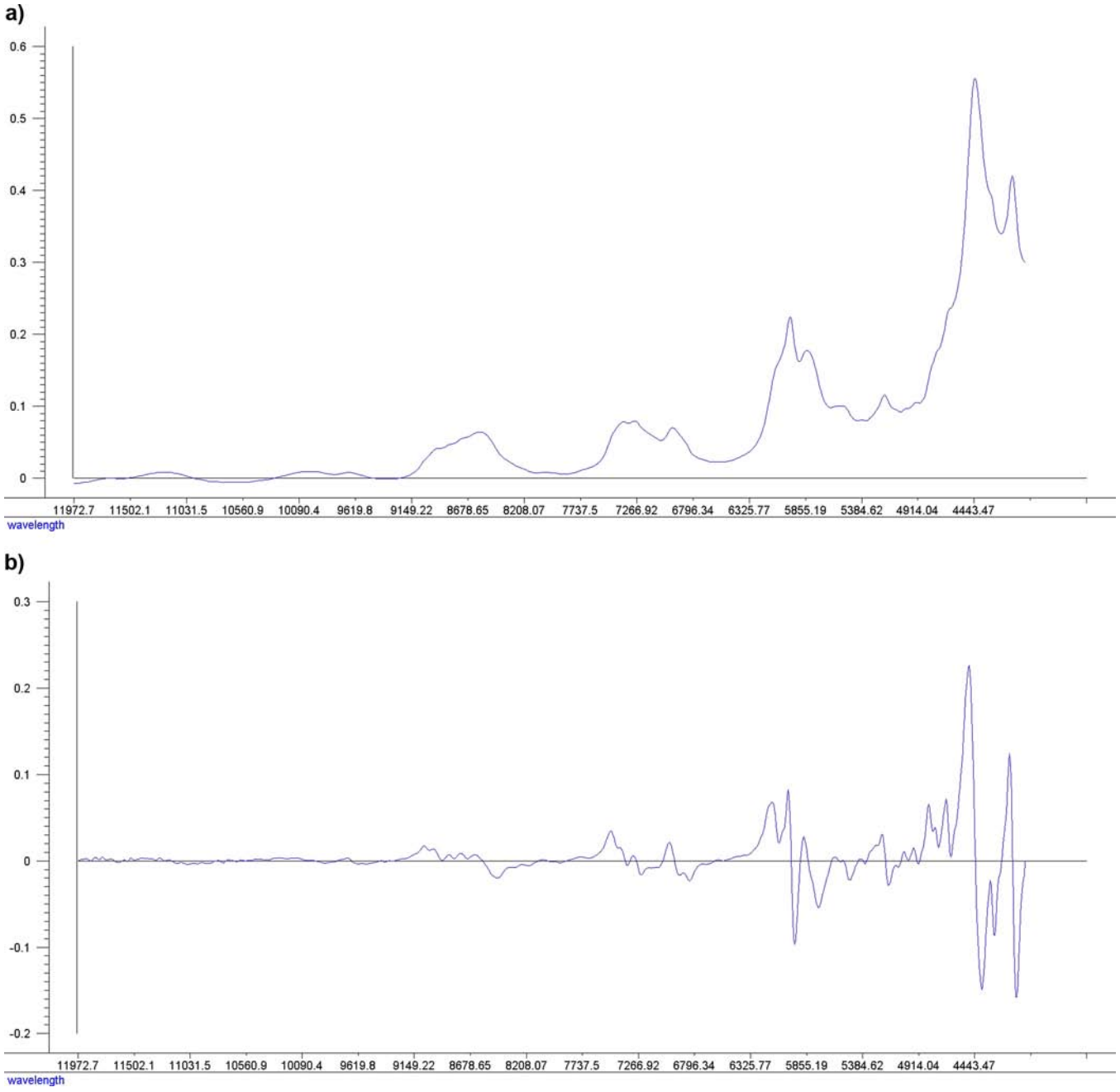

Figure 6. Pure caffeine standard: (a) raw spectra; (b) first-derivative SNV treatment.

calibration.[28] For RPD values >2.5, the calibrations would be suitable for screening lines in breeding programs. For values >5, the calibration would be useful in quality control. For the unground and ground calibrations using both multivariate programs, the RPDs were calculated at >4.0 for unground and ground coffees (Table 3), suggesting reasonably good calibrations for both product types. This was also the case when the decaffeinated coffee was excluded.

The bias for each calibration was also <0.1, which is acceptable on the basis of the suggestion of Shenk, that is, bias < 0.6 × SEC.

Fearn's Statistic. Fearn proposed a calculation to compare if standard deviations of prediction errors and bias between two different calibrations were significantly different when compared to reference values at $P < 0.10$, $P < 0.05$, or $P < 0.01$ levels. Using the Fearn calculation, updated by Reid and Guthrie, there was no significant difference ($P < 0.05$) between the standard deviations of prediction errors and bias for the calibration developed using the two multivariate packages for unground and ground coffee calibrations when the predicted values were compared to the reference values.

NIR Calibrations for Single Beans. The calibrations for the single beans produced high $r^2 > 0.94$ with both multivariate packages with the decaffeinated coffee included. When the decaffeinated coffee was excluded, the Opus program gave a $r^2 > 0.9304$, whereas the Unscrambler package gave a $r^2 = 0.9275$. The r^2 values for cross-validation were >0.91 from both programs when using all five coffees. However, when the decaffeinated coffee was excluded, the r^2 values were considerably lower (Table3).

For single beans, the SECs and SECVs were similar to those for the unground and ground coffee calibrations. The $RPD_{(CV)}$ values were also similar to those of the unground and ground coffee RPDs with the calibrations with all five coffees included being higher than the RPDs for calibrations with the decaffeinated coffee excluded.

The Fearn statistic showed no significant difference ($P < 0.05$) for standard deviation of prediction errors and bias for predicted values from both multivariate packages when compared to the reference values.

Loading Plots/Wavelengths Associations. The raw spectra and first-derivative SNV spectra for the pure caffeine are shown in Figure 6, panels a and b, respectively. The structure of caffeine is shown in Figure 1, with obvious C═C, C═N, O═C—NR, and CH_3 bonds. These combination bonds are strongly associated with regions beyond 5000 cm (2200 nm). The Opus loading plots from the calibrations for bulk unground and ground coffees and single beans are shown in Figure 7, panels a, b, and c, respectively. These loading plots show a number of strong associations in regions associated with proteins and oils. In particular, there are a number of regions associated with NH amino acid groups and urea and C═O associated with oil groups.

DISCUSSION

The development of a NIRS calibration to predict caffeine content in dried coffee has been reported previously and using more samples than in this study.[11,29] However, those studies used a relatively small number of samples but expanded the calibration set by either having samples from multiple locations or by collecting samples from different processing techniques, respectively. Our calibration used only 28 samples but included 2 samples from Australia, which is the first time caffeine content has been reported for Australian-grown coffee. These samples were within the average range of caffeine when compared to international samples.

The novel aspect of our research was the development of a single-bean NIRS calibration to predict caffeine concentration. We believe this to be the first report for a NIR calibration to predict caffeine concentration in single coffee beans. Even with the five different coffees used for the NIRS calibration, there was considerable variation in caffeine concentration. The variation in caffeine concentration was not dependent upon bean weight as only around 30% of caffeine concentration was correlated to bean weight, which indicated selecting for bean weight would not enhance selecting for caffeine concentration.

Our results for unground bulk and ground bulk coffee for caffeine concentration were similar to those of other researchers, who reported r^2 values >0.9, with SECV or SEP around 1−2 mg/g. The potential application values of unground and ground bulk calibrations were similar, based on the RPD values, despite the low number of samples in our calibrations and the use of only a cross-validation approach. The ground coffee calibrations were the best models from both multivariate packages on the basis of the reduced and more uniform particle size. However, in the practical sense, the potential uses of NIR for any coffee trait would be of more value for the prediction and sorting of unground beans. As part of a quality control program at packaging facilities where beans are ground and undergo vacuum-seal packaging, then calibrations for ground coffee could be used.

The calibrations developed used a number of spectral regions associated with the major bonds in caffeine (C═O, C═C, O═C—NR). These molecular bonds are also in protein and oil, which make up around 30% in coffee, so it could be expected to see these regions used in the calibration. In the spectra for pure caffeine, these regions also appeared as major peaks on a 1d spectrum. There are a number of regions identified that were associated with urea O═C—$((NH_2)_2)$. The C═N bonds have also been identified in a study of hydrogen cyanide (HCN) in forage sorghum.[30]

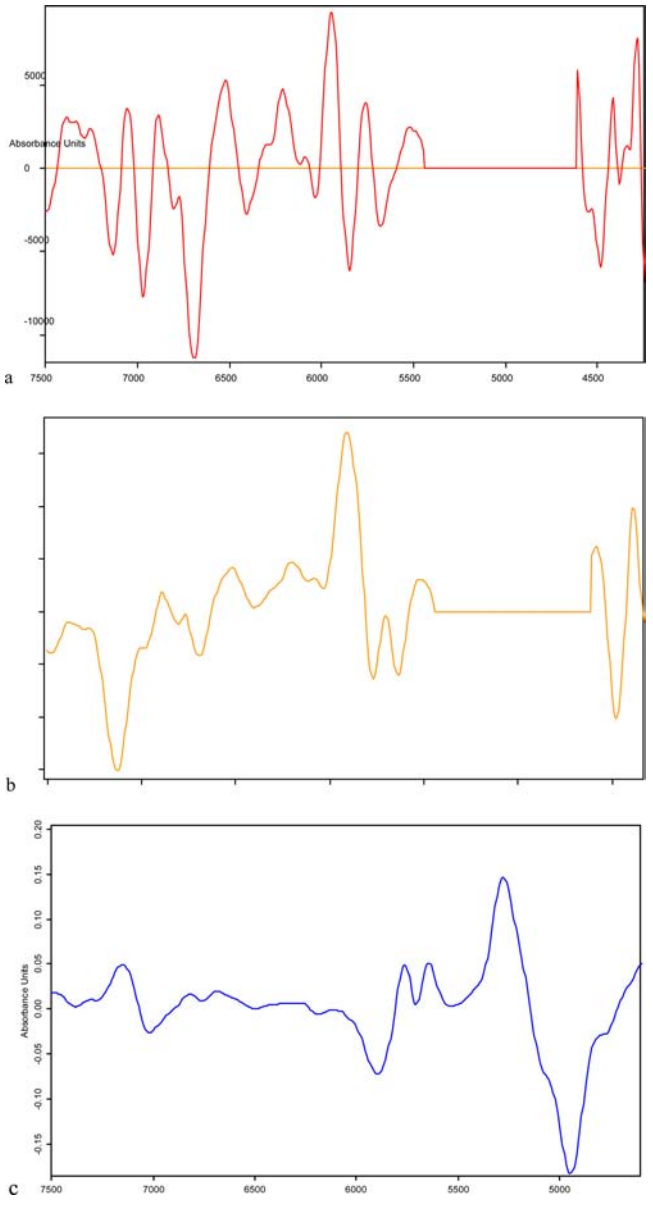

Figure 7. Loading plots for (a) bulk unground, (b) ground, and (c) single beans.

In our study, we compared two multivariate programs, one being the software of the FT-NIR instrument and the other being a commercial package that can use any type of multivariate data. There were some differences in the final calibrations developed using each package. However, as a real world application, using the software dedicated to operating the instrument is a better option. Exporting data into dedicated multivariate packages provides the scope to understand how good a calibration could be.

The development of a NIR calibration to predict caffeine concentration in single coffee beans has shown the potential to

understand the variation in pure Arabica coffees or blended coffees. In addition, to produce a decaffeinated coffee, the caffeine is removed from beans usually by chemical extraction. Using a nondestructive technique such as NIRS could assist processors in knowing the in situ concentration of caffeine in individual beans, which could then in turn help optimize the decaffeination process. Alternatively, this tool could be used to identify and sort naturally low (or high) caffeine beans to develop new coffee products and open new markets.

Although caffeine may not contribute to the major flavor (bitterness) of coffee, consumers purchase coffee with some anticipation of a stimulating effect from caffeine. On the basis of our results, it would be possible to build a calibration to sort beans on caffeine concentration. However, current commercial NIR sorting technology is quite limited in applications and costly, and further research and development are required.

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Notes

The authors declare no competing financial interest.

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