RESEARCH ARTICLE

IR spectroscopy coupled with chemometrics used as a simple and rapid method to determine the caffeine content of tea products

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Abstract

Introduction: Tea is a popular beverage that comes from Camellia sinensis. Tea is generally categorised into four types: black tea, oolong tea, green tea, and white tea. These four types are distinguished based on the presence or absence of a fermentation process during their processing. One of the compounds that play a role in providing freshness to tea is caffeine. **Aims:** The purpose of this study was to determine the caffeine content in the tea samples that are on the market. **Methods:** This was done using the near-infrared (NIR)-chemometric method and using the TLC-Densitometry method as a comparison. Infrared (IR) spectroscopy combined with chemometrics has been developed as a simple method to analyse the caffeine content in a tea sample. IR spectra of tea samples were correlated with caffeine content using chemometrics. **Results:** In this study, the partial least squares (PLS) model of the NIR model that showed the best calibration with r-square was 0.958, and the root mean squared error of calibration (RMSEC) value was 0.070. The PLS calibration model of the NIR models was further used to predict the unknown caffeine content in commercial samples. The significance of the caffeine content that had been measured with NIR and TLC-Densitometry was evaluated using a two paired sample t-test. **Conclusion:** The caffeine content measured with both methods gave no significant difference.

Introduction

The leaves and shoots from tea plants are used to make drinks. There are four types of tea that are distinguished from each other based on their fermentation process. Black tea is fermented, while green tea is not. Oolong tea is made through a partial fermentation process (semi fermentation), and white tea is made by taking the youngest tea leaves, which are immediately evaporated and dried without using a fermentation process first (Hirthe *et al*., 2007).

Consumption of tea can provide general benefits to reduce fatigue, increase physical endurance and mental alertness, as well as by playing a role in the body's recovery process. People often consume tea for health and beauty purposes. Green tea is a type of tea that can be used for weight loss, and it also inhibits premature ageing (Sudaryat *et al*., 2015).

Caffeine is a methylated xanthine derivative alkaloid. The safe limit of caffeine consumption, according to BPOM (Indonesian Food and Drug Administration), is 150 mg/day, which is divided into at least three doses (BPOM, 2003). If caffeine is consumed in the right amount, the body will obtain the benefits. However, if caffeine is consumed excessively, this can trigger heart rate acceleration, feeling nervous or anxious and can even trigger insomnia. Thus, special attention to the levels of caffeine consumed must be taken by people whose bodies are lacking in caffeine tolerance, such as children, adolescents, and pregnant women (Belay *et al*., 2008).

Based on the side effects that arise if caffeine is consumed excessively, it is important to determine its content within tea products on the market. There are several methods that can be used to determine caffeine content, such as UV-Vis Spectrophotometry (Belay *et al*., 2008; Hasanah *et*

al., 2016; Navarra et al., 2017)), High-Performance Liquid Chromatography (HPLC) (Bae et al., 2015; Cunha et al., 2015; Jiang et al., 2015), and TLC-Densitometry (Ford et al., 2005; Riswanto et al., 2015; Torres et al., 2015; Trianto et al., 2009). These methods have several disadvantages, namely requiring specific solvents and reagents. Researchers are currently being challenged to develop an alternative method, which is both fast and reliable. One method that has the potential to be used is near-infrared (NIR) spectroscopy as it has an easier sample preparation process, which does not involve any additional solvents or reagents (Roman et al., 2011; Shafirany et al., 2018).

The spectrum produced by NIR spectroscopy is quite complex, so it is difficult to interpret. To overcome this problem, a method known as chemometrics is needed. Chemometrics uses statistical and mathematical approaches to find the relationship between spectra data and chemical parameters of substances that are difficult to measure directly. This study aimed to create a calibration model using a NIR-chemometric technique in order to determine caffeine content from commercial tea products.

Methods

The materials used were commercial tea products (black tea, oolong tea, green tea, and white tea), caffeine standard, methanol, chloroform, and thin-layer chromatography (TLC) plates. The instruments used were a n°60 sieve, a chamber, an ultrasonicator, micropipette capillaries, a CAMAG densitometer, a NIR device (Brimrose Luminar 3070), and The Unscrambler X 10.2 software.

Preparation of simulation sample

The tea sample simulation was divided into a training set and a test set collected from various shops, shopping centres, and traditional markets in Jember, East Java, Indonesia. Twenty tea product simulations were prepared as training sets consisting of three types of tea (black tea, green tea, and oolong tea). Four tea samples were prepared as test sets that consisted of black tea, oolong tea, and white tea, and three samples were used as the real samples. The samples used in the study can be seen in Table I.

Preparation of caffeine standard

50mg and 30mg of caffeine standard were weighed and then diluted in 25 ml of methanol. The standard caffeine mother solutions had concentrations of 2000 µg/ml and 1200 µg/ml, respectively. The 2000 µg/ml stock solution was diluted to concentrations of 500 µg/ml, 800 µg/ml, and 1000 µg/ml with methanol. Meanwhile, the stock solution (1200 µg/ml) was diluted to become 300 µg/ml and 600 µg/ml.

Table I: Code names used for the tea samples

No.	Code	Brand name of tea samples	No.	Code	Tea samples
(1)	PC	Poci	(15)	BTG	Cap Botol
(2)	SR	Sariwangi	(16)	SSG	Sosro
(3)	SS	Sosro	(17)	JWG	Jawa
(4)	DD	Dandang	(18)	2TG	2 Tang
(5)	KJ	Kepala Djenggot	(19)	XNO	Xiamen
(6)	GD	Gardoe	(20)	GLG	Galan 999
(7)	NG	Naga	(21)	TJ	Tong Tji
(8)	BD	Bandulan	(22)	ZTW	Zet White Tea
(9)	BT	Cap Botol	(23)	GG	Galan 999
(10)	PR	Prendjak	(24)	GP	Gopek
(11)	SM	Sarimurni	(25)	DD2	Dandang
(12)	SB	Cap Sepeda Balap	(26)	SS2	Sosro
(13)	CT	Tjatoet	(27)	XOO	Xiamen
(14)	IDG	Indomaret			

Determination of caffeine content by TLC-Densitometry method

The level of caffeine in the tea samples was determined using the comparison method (TLC Densitometry). The samples weighed 400 mg each, and this process was then replicated three times. The tea sample was diluted into 10 ml of methanol. The sample was inserted into an ultrasonicator and run for 10 minutes. The sample solution was then left to stand for 24 hours in the refrigerator in order to optimise caffeine for extraction and then filtered into the vial using filter paper.

All standard caffeine with sample solutions were put on the TLC plate using a capillary micropipette to measure out 2 µl. After the spot results were dried, the TLC plate was inserted into the chamber, which had been saturated by the chloroform: methanol (9.5:0.5). After the eluent had reached the limit, the plate was lifted and dried. The stain from eluation was scanned, and the purity of the spectra was produced using CAMAG densitometers. The caffeine content in the tea samples was calculated based on the scanning data.

Determination of calibration model

The NIR and Fourier-transform infrared (FTIR) spectra data of the training set samples were analysed quantitatively using partial least squares (PLS), principal component regression (PCR) and support vector regression (SVR) chemometrics through The Unscrambler X 10.2 software. Variable X (predictor) is an absorbance value of the infrared spectrum data that was correlated to variable Y (reference), which represented the caffeine value (% w/w) that was previously determined using the TLC-densitometry method as a comparison. The r-square value of 0.91 or greater and the smaller root-mean-square error (RMSE) value of the model indicated that the model chosen had the best predictive ability. The best calibration model was then tested by leave-one-out cross-validation (LOOCV) and 2-fold cross-validation (2-FCV) techniques. The model was validated by LOOCV by removing a set of sample data from the training set, and the rest of the data were used to create the new model. The 2-FCV was evaluated using the test set as an independent sample.

Application in a commercial sample

The spectra of commercial tea samples were determined by IR spectroscopy. The selected and validated model was then applied to the real sample. The caffeine content of the TLC-Densitometry method results was compared to the IR spectroscopy prediction of the caffeine sample. The results were then inputted into the SPSS Trial version 23.0 programme for further analysis.

Results and discussion

Samples were given identities using a code that was adjusted to each tea sample brand. The results of NIR and FTIR spectrum data are shown in Figure 1. The spectra data of all samples were used as predictors of the calibration model.

Determination of caffeine content by TLC-Densitometry

The wavelength used to scan the spots on the TLC plate using the densitometer was 277 nm. The caffeine content from each sample was expressed using % w/w. The level of % w/w of each tea sample was determined by converting the concentration value of the analyte that appeared in the form of a nanogram unit on the densitometer scan results to the initial weighing and dilution. The caffeine content obtained is displayed in Table II.

Table II: Results of R-square and RMSE values of the NIR model

Model	R-square Calibration	R-square Validation	RMSEC	RMSECV
PLS	0.9579	0.9579	0.0699	0.0663
PCR	0.6219	0.6078	0.2108	0.6054
SVR	0.5597	0.5243	0.2300	0.2175

Determination and validation of the calibration model

Three hundred twenty spectrum data values from NIR and 58 spectrum data values from FTIR were analysed quantitatively using chemometrics on the Unscrambler X 10.2 software. The spectra were analysed using the PLS, PCR and SVR techniques. The spectrum of the training set sample was correlated with the caffeine concentration for determining the calibration model. Spectra data as the variable y (response) and the value of caffeine content of the results of TLC-Densitometry expressed by % w/w as the variable x were used to predict the variable y (predictor). The best calibration model was the PLS model using NIR spectra because its R^2 and RMSEC values were 0.9579185 and 0.0698975, respectively (Table II). The calibration model showed a good R-value (above 0.91) (Lengkey *et al.*, 2013) and a good RMSEC value. Thus, if the value gets smaller, it will also produce a better calibration model as it shows predictive results that are the same as or near the actual concentration (Trianto *et al.*, 2009). The slope parameter shows the average increase or decrease in the Y variable for an increase in one variable X (the slope size of a line). If the slope is positive, then the shape of the line will increase to the right, as shown in the results of this study. This value indicates that the PLS model of NIR has formed good regression linearity, which was the actual value, and the predictive value has a close correlation (Lengkey *et al.*, 2013).
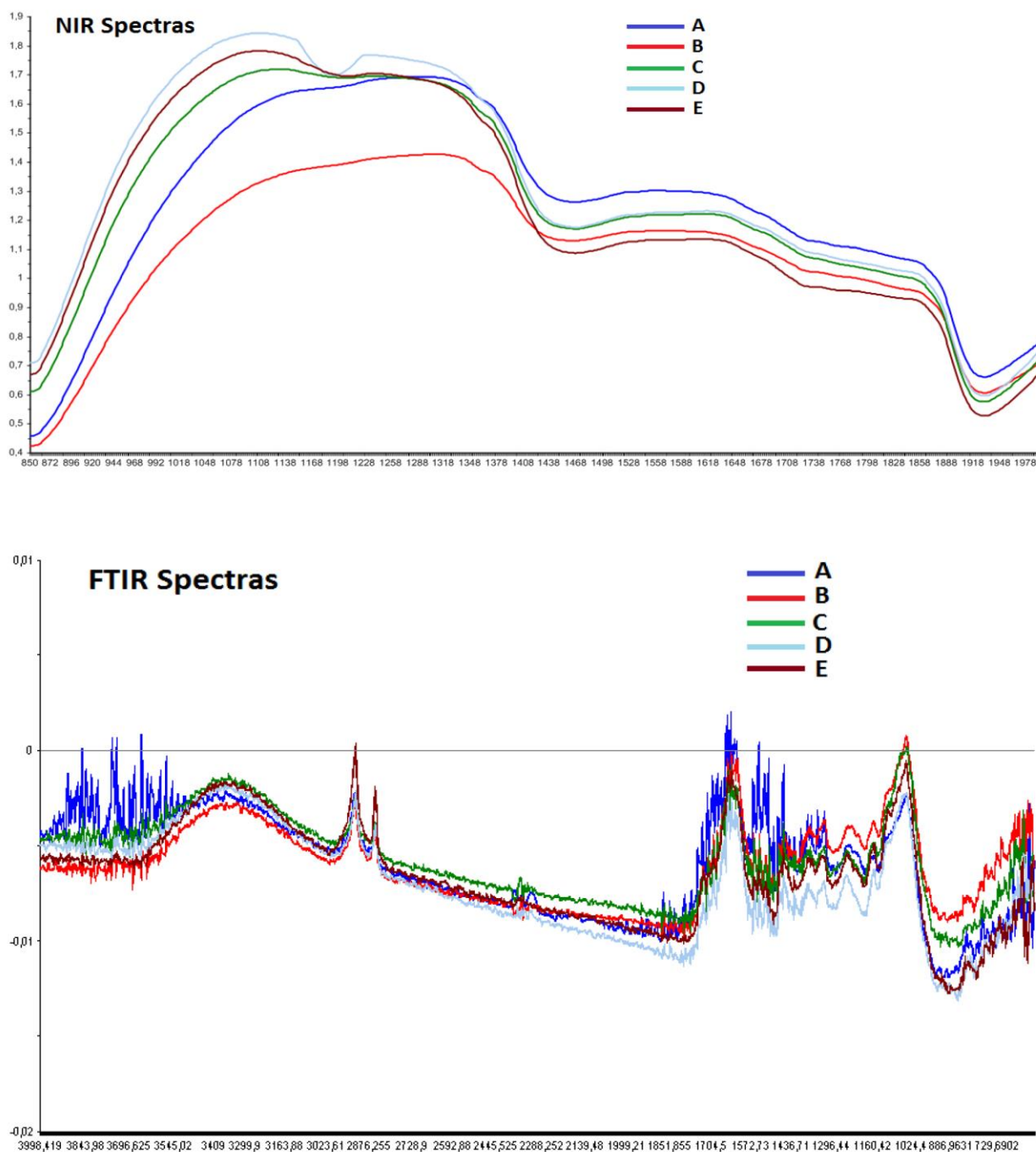
Figure 1: Spectra of NIR and FTIR, identity sample DD (A), KJ(B), PC (C), PR (D), XNO (E)

The calibration model using FTIR spectra had an R2 below 0.91, so it was the only calibration model using NIR spectra that was further validated. The results of the LOOCV PLS model of NIR showed that the R-square value was greater than 0.91, and the RMSE values were small (±0.06) (Table III). Meanwhile, the results of 2-FCV validation through the prediction of the test set sample showed that the R-square and RMSE values obtained were 0.9250847 and 0.0909219, respectively. Based on the results of LOOCV and 2-FCV validation, it can be concluded that the reliability or consistency of the prediction ability of the PLS calibration model NIR was well-formed, so it can be implemented in the actual sample.

Table III: Results of LOOCV PLS calibration model NIR

Number	Sample leaved	RMSE	R-Square
1	BD	0.0645	0.9486
2	JWG	0.0624	0.9478
3	XNO	0.0679	0.9432
4	SM	0.0660	0.9493

The PLS was validated as the best model calibration and was then applied to the real sample. The real samples that were obtained were scanned using a NIR spectrophotometer, and then the caffeine samples were determined using TLC-Densitometry. The caffeine content of TLC-Densitometry results was compared to the NIR scan caffeine levels by inputting both levels of data into the SPSS trial version 23.0 programme for further analysis. The mean value of % w/w caffeine resulting from the NIR spectroscopy method was compared to the results of the TLC-Densitometry method (Table IV).

Table IV: The comparison results from the NIR spectroscopy and the TLC-Densitometry

Sample code	Mean value %bw ± SD	
	NIR spectroscopy	TLC-Densitometry
DD2	1.28 ± 0.03	1.33 ± 0.01
SS2	1.52 ± 0.02	1.53 ± 0.03
XOO	1.33 ± 0.09	1.36 ± 0.03

Two paired t-test samples were used for the analysis in order to obtain information about whether there was a significant difference between the levels of real samples of NIR scan results with TLC-Densitometry. The results of the analysis of the two paired sample t-test showed that the significance value produced was 0.122 (> 0.05), so Ho was accepted. This meant there was no significant difference between the caffeine levels of real samples obtained from NIR and TLC-Densitometry.

Conclusion

The infrared spectroscopy method in the PLS calibration model of NIR spectroscopy-coupled with the chemometrics method can be used to determine caffeine content. The results of determining the caffeine content in both NIR Spectroscopy and TLC-Densitometry methods are the same or have no significant differences. The IR spectroscopy method is rapid, precise, accurate and eco-friendly.

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