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Utilization of UV-visible and FTIR spectroscopy coupled with chemometrics for differentiation of Indonesian tea: an exploratory study

Mitsalina Fildzah Arifah[1], Agustina A.M.B. Hastuti[1,2], Abdul Rohman[1,2]*

1. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.
2. Center of Excellence Institute for Halal Industry and Systems (PUI-PT IHIS), Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

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*Corresponding author
Abdul Rohman

Email:
abdul_kimfar@ugm.ac.id

ABSTRACT

Ultraviolet (UV)-visible and Fourier-transformed infrared (FTIR) spectroscopy are two of the most popular and readily available laboratory instruments. Fingerprinting analysis of the UV-visible and FTIR spectra has been applied for food classification and authentication studies. In this study, the UV-visible and FTIR spectra of brewed tea, and their data fusion data sets, were used to build models for the classification of tea based on tea types and origins. The study included black and green tea samples from several provinces in Sumatra and Java Islands (Indonesia). Chemometric models of principal component analysis (PCA), k-nearest neighbor (kNN), and logistic regression were developed for classification purposes. All PCA models were able to well-separate the tea groups. kNN and logistic regression models based on UV-visible spectra successfully classified green and black tea with >0.8 classification accuracy. The kNN model of FTIR spectra had good accuracy (0.903) for classifying tea based on its origin. ReliefF algorithm was employed to select the best features among the data fusion data sets of UV-visible and FTIR spectra. The data fusion data sets of UV-visible and FTIR spectra demonstrated good separation of tea types and origins with a high area under the receiver operating characteristic (ROC) curve (>0.8) and moderate accuracy (0.548). Therefore, UV-visible and FTIR spectroscopy may provide complementary information for tea classification based on tea types and origins.

Keywords: tea, Indonesia, FTIR, UV-visible spectroscopy

INTRODUCTION

Tea, derived from the leaves and buds of *Camellia sinensis* L., is one of the most popular beverages in the world. Indonesia is a major tea-producing country worldwide, with 109,938 hectares of tea plantation area and 140,237 tons of production volume in 2018. Tea as a commodity is produced in ten provinces in Indonesia, spread in Sumatra and Java Islands. National consumption accounts for almost two-thirds of Indonesian tea production. The remaining tea is exported as black tea and green tea (Badan Pusat Statistik - BPS-Statistics Indonesia, 2019).

The post-harvesting process of tea leaves determines the type of tea. Full-fermentation of tea leaves using enzymatic oxidation during the post-harvesting process produces black tea. On the other hand, green tea does not undergo the enzymatic oxidation process so that the scent of the leaves is preserved (McKenzie *et al*., 2010). Both black and green tea contain high levels of polyphenols. The most abundant polyphenols in green tea are epigallocatechin and its derivatives, while theaflavin derivatives dominate the polyphenols in black tea (Seow *et al*., 2020). The polyphenol content in tea is commonly associated with its health benefits, such as antioxidant (Xu *et al*., 2020), anti-inflammatory, anticancer (Musial *et al*., 2020), antiviral (Mhatre *et al*., 2021), and reducing neurodegenerative risks (Kakutani *et al*., 2019). Besides polyphenols, tea also contains caffeine, amino acids, polysaccharides, carotenoids, and alkaloids, among other compounds (Ma *et al*., 2018).

Fingerprinting analysis has been gaining much popularity in food and herbal authentication and quality control in recent years. Spectroscopic and chromatographic techniques produce unique spectra and chromatograms, respectively, that contain more wholesome information on the characteristics of a product. Chemometrics is necessary to extract the information derived from the spectra or chromatogram, as the data collected is extensive. Seasonal, geographical, chemotaxonomic, post-harvest processing, and other factors can be identified using chemometrics on the spectra or chromatogram (Kharbach *et al*., 2020). Fourier-transformed infrared (FTIR) spectroscopy was applied to analyze the polysaccharide extracted from tea leaves. The model derived from partial least squares (PLS) and self-organizing map (SOM) neural network on the pre-processed FTIR spectra was able to distinguish tea of different varieties (Cai *et al*., 2015). Ultraviolet (UV)-visible spectroscopy method was also used to characterize the water extract of tea leaves (Wang *et al*., 2013). As for chromatographic methods, two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOF-MS) was utilized to characterize water extract of various types of tea. Fingerprinting analysis of 74 analytes of at least seven chemical classes successfully distinguished many tea types (oolong, puerh, and black tea) that were less affected by seasonal effects (Stilo *et al*., 2020). Ethanolic extracts of tea leaves were subjected to high-performance liquid chromatography (HPLC) for quality assessment of green tea by comparing ten observed peaks with standard chromatographic fingerprints (He *et al*., 2015).

In the context of whole-tea classification based on geographical origin and tea type, UV-visible and IR spectroscopy have been used to classify tea from various countries on different continents, none of which are from Indonesia (Aboulwafa *et al*., 2019; Dankowska & Kowalewski, 2019; Diniz *et al*., 2016). This study used tea samples exclusively from Indonesia for classification purposes. The use of UV-visible and FTIR spectroscopy, aided with chemometrics, was investigated to discriminate between different types of tea depending on the post-harvesting process, which included black tea and green tea. The tea classification based on geographical origins of different islands in Indonesia was also explored. The UV-visible and FTIR spectroscopy are easily suitable for authentication or classification studies of various plants or herbals due to simple sample preparation processes and generally accessible UV-visible and FTIR spectroscopy equipment.

MATERIAL AND METHODS

Samples

Thirty-three single-origin tea samples from plantations in seven tea-producing provinces in Indonesia were purchased directly from legitimate sources, such as PT Perkebunan Nasional (Indonesian National Plantation Company who has tea plantations in different provinces), premium tea industries who have their own specific plantations, and farmers from the tea plantation. The samples consisted of 25 black tea and 8 green tea (Figure 1). The samples were preserved in their original packaging and kept in a cool and dry place away from sunlight.

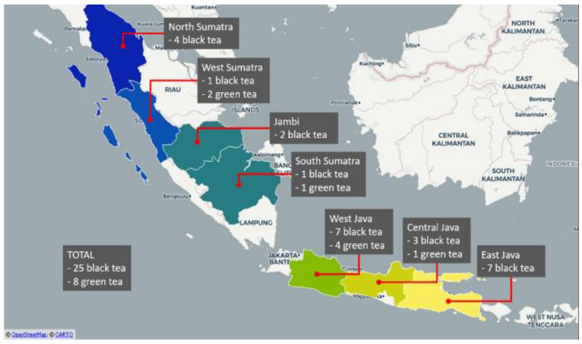

Figure 1. The origin of the tea samples used in this study. A total of 25 black tea and 8 green tea samples were collected from 7 tea-producing provinces in Indonesia. Red dots indicate the province but not the exact location of the tea plantation area. The map is derived from OpenStreetMap processed with Orange v3.29.3 software.

Sample preparation

Approximately 1 g of tea was accurately weighted. The tea was brewed with 80 ml of hot water (90-95°C) for 5 minutes. The brewed tea was immediately filtered with a Hario VCF-01-100W paper filter (Hario, Tokyo, Japan). After reaching room temperature, the filtrate was added with water to a volume of 100 ml using a volumetric flask. The sample was diluted ten-fold for UV-visible spectral acquisition, while no dilution was performed for FTIR analysis. The samples were kept refrigerated at 4°C before analysis. The absorbance measurements were conducted within two days of the sample preparation to ensure the freshness of the sample. Distilled water (General

Labora, Yogyakarta, Indonesia) was used throughout the experiment.

UV-visible spectral acquisition

The UV-visible spectral acquisition was conducted using a Hitachi U-2900 double-beam spectrophotometer equipped with a deuterium lamp for the UV region and a tungsten-iodine lamp for the visible region (Hitachi, Tokyo, Japan). Ten mm quartz cuvettes were used to hold the sample and the blank. Distilled water was used as the blank solution. The wavelength scanning was performed at 190-500 nm at every 1 nm, with a measurement speed set at 400 nm/min. The baseline autocorrection was done before every sample measurement.

FTIR analysis

FTIR absorbance was measured using a Thermo Nicolet iS10 FTIR spectrometer with a Smart iTR diamond ATR sampling accessory (Thermo Fisher Scientific, Madison, USA). The FTIR scanning was performed from 4000 cm^{-1} to 650 cm^{-1} at every 0.964 cm^{-1}. The number of scans was 32, with a resolution of 8. Automatic atmospheric suppression was applied to remove H_2O and CO_2 interference. The sample window was cleaned with acetone (General Labora, Yogyakarta, Indonesia) and Kimwipes (Kimberly Clark Professional, Georgia, USA) before and after each sample. The background collection was performed before every sample measurement.

Data analysis

The sample absorbance of each datapoint in the UV-visible spectra was normalized to the weight of the tea samples for further data processing. The UV-visible and FTIR spectra were subjected to PCA for explorative purposes. Because PCA demonstrated that UV-visible and FTIR spectra clustered data according to tea type and origin, respectively, the ReliefF method was used to choose the best features of the associated spectra. One-hundred-fifty variables from each UV-visible and FTIR spectrum that best-explained tea types and origins, respectively, were selected using the ReliefF algorithm. The resulting data fusion data sets were subjected to PCA. The classification models on UV-visible spectra, FTIR spectra, and data fusion data sets were built using supervised methods of k-nearest neighbor (kNN) (non-parametric) and logistic regression (parametric). The kNN model was created by measuring five nearest neighbors using Euclidean distance and uniform weight. The logistic regression was performed using ridge (L2) regularization with C=1 (moderate) strength. Leave-one-out cross-validation was used to validate the models. Orange v3.29.3 was used to analyze the data (University of Ljubljana, Ljubljana, Slovenia).

RESULTS AND DISCUSSION

UV-visible and FTIR spectra

Brewed black and green tea yielded UV-visible spectra with identical shapes ranging from 190 nm to 500 nm (Figure 2). The peak in UV-visible spectra is broad and unspecific, mostly influenced by $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions of chromophores and auxochromes in tea constituents. The pattern of UV-visible spectra correlates with flavonoids and methylxanthine compounds found in black and green tea, such as caffeine (maxima at 280 nm (López-Martínez *et al.*, 2003)), catechin and related compounds (maxima at 275 nm (Sarkar *et al.*, 2014)), and other phenolic compounds (Boulet *et al.*, 2017).

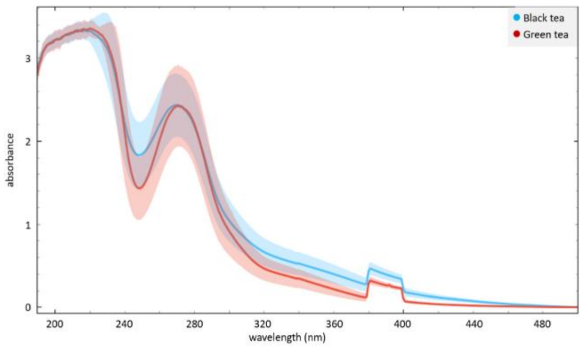

Figure 2. The UV-visible spectra of brewed tea. The darker color indicates the average of the spectra within each group (blue = black tea, red = green tea).

On average, the UV-visible spectra of black tea had higher absorbance at minima of approximately 248 nm and 300-500 nm. The higher absorbance of black tea at around 380 nm was also observed in the UV-visible spectra of methanolic extract, associated with a higher concentration of theaflavins and thearubigins (Palacios-Morillo *et al.*, 2013; Roberts & Smith, 1961). Theaflavin and its galloyl esters are the oxidation products of catechins by polyphenol oxidase or peroxidase. Thus, it is present in black tea but not in green tea, in which the endogenous enzymes have been inactivated during the post-harvesting process (Takemoto & Takemoto, 2018).

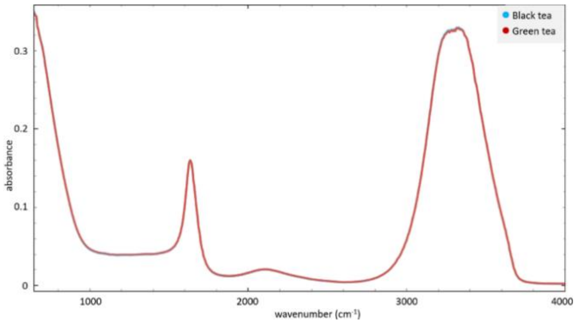

Figure 3. The FTIR spectra of brewed tea. The spectra of black and green tea completely overlap. Blue = black tea, red = green tea.

Unlike the UV-visible spectra, the FTIR spectra of black and green tea were indistinguishable, with only three peaks observed due to the presence of water solvent (Figure 3). A broad peak of O-H-stretching modes around 3100-3600 cm^{-1} indicated the presence of many phenolic compounds in tea. Weaker N-H-stretching mode peaks, as well as C-H saturated and unsaturated peaks, might be present in this area but are obscured by the peak's broadness. A modest peak at 2000-2200 cm^{-1} might indicate a C≡C bond and/or a C≡N bond. A reasonably sharp peak at 1634 cm^{-1} indicated the presence of a carbonyl functional group, most likely an amide group. However, C=O peaks from other carbonyl groups might overlap in this region. The spectral pattern reflects the tea constituents, as discussed earlier.

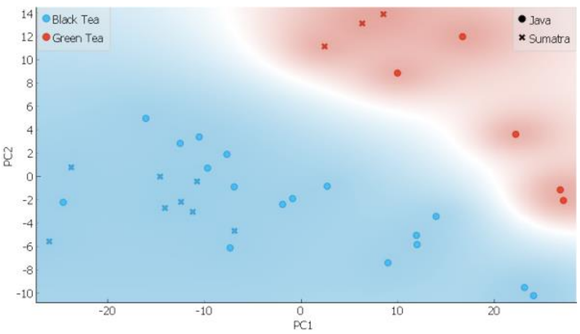

Figure 4. The PCA scores plot of the UV-visible spectra of brewed tea. Colors correspond to tea types (blue = black tea, red = green tea), while shapes correspond to sample origins (circle = Java, cross = Sumatra).

PCA

The unsupervised learning algorithm of PCA was used to explore the native ability of UV-visible and FTIR spectra to classify the tea samples. The PCA model constructed from the UV-visible spectra of brewed tea samples identified distinct groups of tea types (Figure 4). The sample variance was well covered, with PC1 explaining 75.5% of the total variance and PC2 explaining 12.0% of the total variance. As a result, the PCA model provided a good approximation of the variation in the tea samples. The absorbance around 250 nm and from 350 to 490 nm had the greatest effect on the PC1, reflecting the difference in the UV-visible spectra of black and green tea. The Java and Sumatra tea samples, while not fully separated, were also clustered in a relatively similar location. However, the provinces of origin did not significantly contribute to sample grouping (Figure S1).

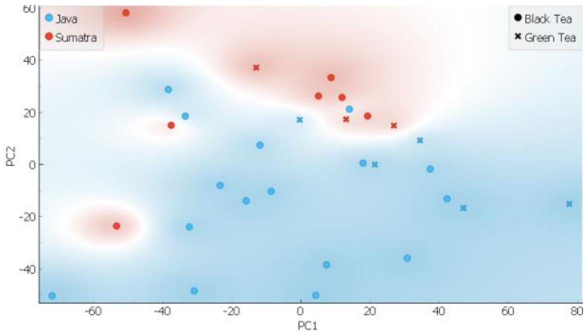

Figure 5. The PCA scores plot of the FTIR spectra of brewed tea. Colors correspond to sample origin (blue = Java, red = Sumatra), while shapes correspond to tea types (circle = black tea, cross = green tea).

In PCA generated from FTIR spectra of brewed tea samples, two of the spectra were considered outliers and removed from further analysis (Figure S2). The resulting PCA model showed good separation of Java and Sumatra tea (Figure 5). PC1, PC2, and PC3 explained 31.4%, 21.1%, and 8.7%, respectively, of the total variance. PC1 was mostly loaded by the peak at 1250-1450 cm^{-1} and 2850-3000 cm^{-1}, while PC2 was mostly loaded by the peak at 1600 cm^{-1} and 3500-3650 cm^{-1}. The peak at 3400 cm^{-1} strongly influenced PC3. Thus, all peaks observed in the FTIR spectra contributed to the grouping in the PCA model. Different soil and climate characteristics in Java and Sumatra, evidenced by seasonal evolution and interannual variability of the climate along the coasts (Susanto *et al.*, 2001), influenced secondary metabolites produced by plants (Yang *et al.*, 2018). Despite this, the change in the FTIR spectra of the water extract could not be visually observed. As for the tea types, green tea was located at the edge of the black tea group, but no clear grouping was observed. Two of the Sumatra samples that strayed into the Java cluster were Jambi black tea. One Java black tea located in the Sumatra cluster was a Central Java black tea. The provinces of origin might influence the sample groupings to some extent, with West Java and East Java tea forming distinct clusters (Figure S3).

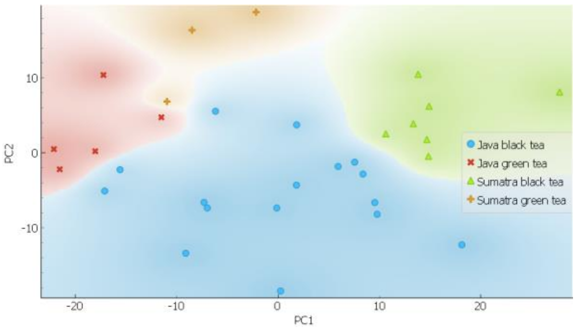

Figure 6. The PCA scores plot of the UV-visible and FTIR spectra data fusion data sets of brewed tea. Colors and shapes correspond to tea types and origins (blue circle = Java black tea, red cross = Java green tea, green triangle = Sumatra black tea, orange plus = Sumatra green tea).

Data fusion data sets of UV-visible and FTIR spectra was employed to construct a PCA model to establish a comprehensive clustering based on tea types and origins. Variable reduction is necessary before building a PCA model because an unbalanced number of variables in UV-visible and FTIR spectra gives different weights, with FTIR spectra having more influence due to a higher number of variables. ReliefF, an algorithm to select the best features, generally has a good overall performance and is sensitive to feature interaction (Urbanowicz *et al*., 2018). In this study, 150 best features in UV-visible and FTIR spectra were selected using the ReliefF algorithm. The best features from ReliefF selection were similar to high-scored-components in PCA model (350-495 nm for UV-visible spectra; 1576-1675 cm^{-1}, 1760-1880 cm^{-1}, and 3460-3550 cm^{-1} for FTIR spectra). The selection of meaningful features, noise reduction, and elimination of redundant variables made the distinction between tea types and origins well observed (Figure 6). PC1 and PC2 explained 56.1% and 22.2% of the variance, respectively. In PC1, most of the UV-visible spectral features had higher loading scores than FTIR spectral features. On the other hand, the FTIR spectral features scored higher than UV-visible spectral features in PC2. Thus, both UV-visible and FTIR spectra were complimentary for the classification of tea based on tea types and origins.

k-nearest neighbor and logistic regression

Analysis of small datasets using complex machine learning suffers from overfitting. In this study, simple machine learnings of both non-parametric (kNN) and parametric techniques (logistic regression) were evaluated using leave-one-out cross-validation to reduce bias. UV-visible spectra generally had good performance with >0.9 area under the ROC curve (AUC), >0.8 classification accuracy, and >0.8 precision (Table I). All black tea was correctly classified, while 50% and 25% of green tea were misclassified in kNN and logistic regression models, respectively (Table II). The kNN model of the FTIR spectra had good accuracy (0.903) but with a low AUC (0.788) as both Java tea and Sumatra tea suffered misclassification. In the logistic regression model of FTIR spectra, all the Sumatra tea were misclassified, resulting in a zero AUC. The kNN and logistic regression of the data fusion data sets of UV-visible and FTIR spectra produced a similar quality of the groupings. The AUCs were relatively high (>0.8) with moderate accuracy (0.548) and precision (>0.4) on both classification models evaluated with leave-one-out cross-validation. Of the three spectral data, kNN and logistic regression showed relatively similar classification performance using UV-visible spectra and data fusion data sets. In FTIR spectra, however, logistic regression showed worse performance compared to the kNN model. Thus, the classification based on FTIR spectra probably did not follow a linear relationship.

When the kNN and logistic regression were applied on three spectral collections, the two classification approaches on UV-visible spectra produced the best classification models for tea types. The data fusion data sets of UV-visible and FTIR spectra could potentially be employed to classify tea types and origins as the specificity and sensitivity, as indicated in AUC, were relatively high. The approach benefited from a rapid and uncomplicated preparation procedure that used less organic solvent (i.e., compared to the chromatographic techniques). Unfortunately, the dataset in this study was too small to be split into a training set and testing set for a prediction study. Further sample collection, especially of the underrepresented samples from Sumatra, is needed to build a more robust model.

Table I. Evaluation results of the kNN and logistic regression models built using UV-visible spectra, FTIR spectra, and data fusion data sets. The models were validated using leave-one-out cross-validation.

	UV-visible spectra	**FTIR spectra**	**Data fusion data sets**
Grouping	Type (black tea, green tea)	Origin (Java, Sumatra)	Type and origin (Java black tea, Java green tea, Sumatra black tea, Sumatra green tea)
kNN			
AUC	0.950	0.788	0.834
Classification accuracy	0.879	0.903	0.548
Precision	0.896	0.903	0.498
Logistic regression			
AUC	0.990	0.000	0.825
Classification accuracy	0.939	0.677	0.548
Precision	0.944	0.459	0.472

Table II. Confusion matrices of kNN and logistic regression models built using UV-visible spectra, FTIR spectra, and data fusion data sets. BT=black tea, GT=green tea, JT=Java tea, ST=Sumatra tea, JBT=Java black tea, JGT=Java green tea, SBT=Sumatra black tea, SGT=Sumatra green tea.

UV-visible spectra

kNN			***Logistic regression***		
Actual	Predicted		Actual	Predicted	
	BT	GT		BT	GT
BT	25	0	BT	25	0
GT	4	4	GT	2	6

FTIR spectra

kNN			***Logistic regression***		
Actual	Predicted		Actual	Predicted	
	JT	ST		JT	ST
JT	20	1	JT	21	0
ST	2	8	ST	10	0

Data fusion data sets

kNN					***Logistic regression***				
Actual	Predicted				Actual	Predicted			
	JBT	JGT	SBT	SGT		JBT	JGT	SBT	SGT
JBT	10	2	4	0	JBT	14	0	2	0
JGT	3	1	0	1	JGT	3	2	0	0
SBT	1	0	6	0	SBT	6	0	1	0
SGT	2	1	0	0	SGT	2	1	0	0

Sumatra tea is not commonly available in the markets as the production capacity is much lower than the Java tea plantation (Badan Pusat Statistik - BPS-Statistics Indonesia, 2019). By acquiring more samples, more complex and powerful machine learning, such as random forest, can be applied to make a better prediction on tea classification.

CONCLUSIONS

This study investigated the use of UV-visible and FTIR spectra of brewed tea, assisted by chemometrics, for the classification based on tea types and origins. PCA, kNN, and logistic regression models of UV-visible spectra satisfactorily classified black and green tea. FTIR spectra for PCA and kNN models showed good classification for Java and Sumatra tea. The data fusion data sets of

UV-visible and FTIR spectra provided complementary methods to classify tea types and origins. However, more samples are still required for model prediction testing. Nevertheless, this exploratory study showed that UV-visible and FTIR spectroscopy coupled with chemometrics are promising tools for the classification of tea collected from a relatively small geographical area (i.e., intra-national).

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