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Detection of Adulterants Metanil Yellow in Turmeric Powder Using Fourier Transform Infrared (FTIR) Spectroscopy combined with Chemometrics OPLS-DA and PLS

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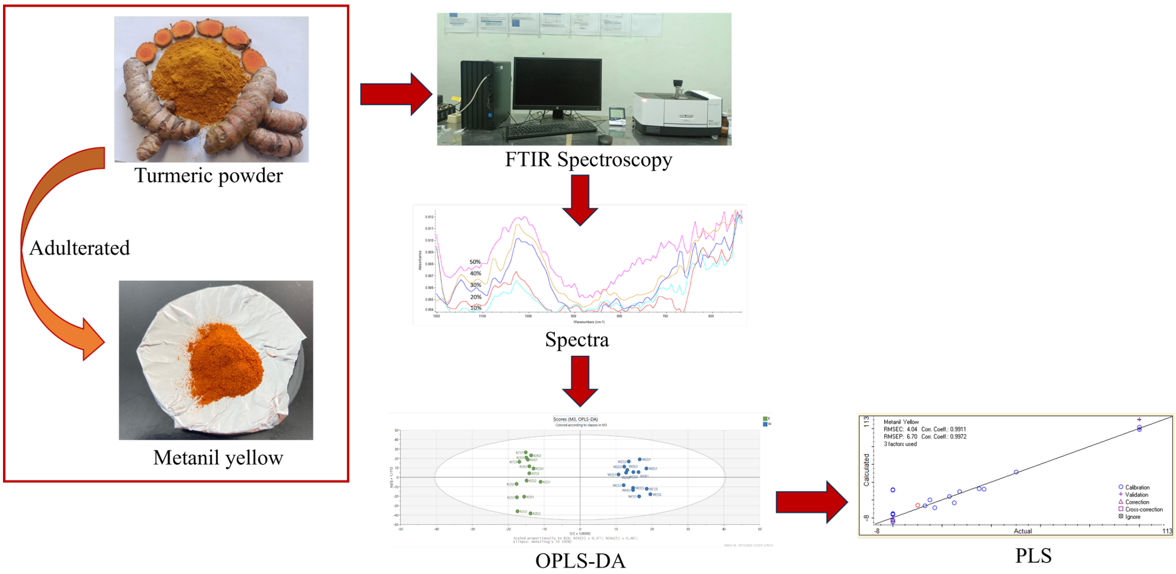
GRAPHICAL ABSTRACT



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ABSTRACT

Turmeric powder (*Curcuma longa L.*) is often used for food coloring and flavoring. The high demand for turmeric powder has led to adulteration, one of which is metanil yellow. This study aims to detect metanil yellow dye in turmeric powder using Infrared Spectroscopy (FT-IR) and Chemometrics. The chemometrics model was built by preparing turmeric powder and metanil yellow in the concentration range of 0-50% (w/w). Data was processed using multivariate calibration using OPLS-DA (Orthogonal Partial Least Squares Discriminant Analysis) and PLS (Partial Last Square). The results obtained from OPLS-DA processing of market samples 1 and 2 are estimated to be pure turmeric powder, and samples 3 to 10 are adulterated. From the PLS results, the best calibration model was obtained at wave numbers 1199-539 cm^{-1} with R^2 values for calibration and validation of 0.9967 and 0.9970, respectively, with RMSEC of 2.48 and RMSEP of 6.35. The PLS results showed that in sample 3 containing metanil yellow, in samples 4 to 10, no metanil yellow was detected. The ingredients added were not metanil yellow. It was concluded that FTIR combined with chemometrics could detect the metanil yellow in turmeric powder.

1. INTRODUCTION

Spices are biological resources taken from parts of plants that have an essential role in human life. They have many benefits, including food flavoring, flavor enhancers, and fragrances. Some are natural food coloring, such as turmeric powder [1, 2]. Turmeric powder was made from dried rhizomes [3]. Turmeric (*Curcuma Longa*) is a perennial herb belonging to the Zingiberaceae family. Turmeric is widely cultivated in tropical and subtropical regions such as Indonesia, India, and China [1, 4-5]. Turmeric consists of 60-70% carbohydrates, 8.6% protein, 5-10% fat, 2-7% dietary fiber, 3-5% curcuminoids (with 50-70% curcumin content), and 5% volatile oil and resin [6-7]. It also contains vitamins and minerals, including vitamin C (ascorbic acid), vitamin E (α-tocopherol), vitamin B3 (niacin), potassium (K), magnesium (Mg), phosphorus (P), and calcium (Ca) [8-9].

Turmeric powder has potential health benefits such as antioxidant activity [10], antimicrobial effects [11], anti-inflammatory properties [12], anti-arthritis effects, anti-viral effects, anti-asthma and anti-diabetic effects, anti-obesity, cardio and liver toxicity protection activity, anti-cancer activity, anti-metabolic syndrome activities, neuroprotective activity, anti-depression, and anxiety activities. Turmeric has been widely used to treat cough, sore throat, and respiratory ailments and could be an effective immunity booster against SARS-CoV-2 therapy during the pandemic [13].

Due to its many benefits, turmeric powder has become a top-rated commercial product, and incidences of turmeric adulteration by different botanical products and chemical dyes have increased for financial gain [14]. Some investigations reported the mixing of *Curcuma zedoaria*, a wild relative of turmeric, into turmeric powder due to its close resemblance with turmeric [15]. In some cases, metanil yellow ($C_{18}H_{14}N_3NaO_3S$) has been added to turmeric powder to improve the appearance of curcumin [16-17]. Metanil yellow classifies as a CII group drug due to its toxicity [18-19]. Oral administration of metanil yellow caused tumors, intestinal problems [20], neuro-toxic hepatocellular carcinoma, lymphocytic leukemia, and other life-threatening diseases [21].

Different analytical methods have been developed to detect the adulteration of spice powder. High-performance liquid chromatography (HPLC), high-performance capillary electrophoresis, HPLC-electrospray, polymerase chain reaction (PCR), Nuclear Magnetic Resonance (NMR), and ionization tandem mass spectrometry are commonly used techniques for authentication of spice powders [15, 22-25]. Most methods use expensive chemical reagents and complex processing of samples, but they are still destructive. Therefore, it is essential to develop efficient and non-destructive methods for analyzing adulterations in turmeric powder [26].

Fourier transform Infrared (FTIR) spectroscopy and chemometrics were widely developed to detect adulteration in spice powder. These methods are faster, easier to use, and non-destructive analytical methods [26]. FTIR spectroscopy with chemometrics was used to detect adulteration of turmeric powder with Sudan red, metanil yellow, and starch [18, 26-27] and adulteration of chili powder with Sudan I dye [28]. Based on the conducted literature review, FTIR spectroscopy has not been applied in conjunction with supervised pattern recognition, predominantly orthogonal partial least square discriminant analysis (OPLS-DA), to detect metanil yellow in turmeric powder.

Hence, this study aimed to detect metanil yellow in turmeric powder using FTIR spectroscopy combined with the chemometrics method. The OPLS-DA was developed to classify metanil yellow, pure turmeric, and commercial turmeric powder based on supervised pattern recognition. Additionally, partial least square (PLS) regression was chosen to establish the adulteration turmeric power prediction models.

2. EXPERIMENTAL METHODS

2.1. Materials

In the present study, some apparatus and materials were used. The apparatus included glassware, an analytical balance, microtube, silica gel 60 F254 plate (Merck, Singapore), micro syringe, oven, blender, and FTIR instrument Qatar-S Single Bounce Diamond ATR (Shimadzu, Japan). Ten commercially available turmeric powders were used, and turmeric roots were collected from Larangan Traditional Market, Candi District Sidoarjo Regency East Java. Metanil yellow (70% dye) was supplied from the local market. The other materials are ethanol (Merck, 90%), toluene (Merck, >99.9%), and acetic acid glacial (Merck, 100%).

2.2. Sample preparation

The procedure used to prepare pure samples (turmeric powder with a 100% purity level) was based on the published method [29]. The pure samples were prepared by washing the roots properly and then dried in an oven at 70 °C. After drying, the roots were chopped in a blender for about 15 minutes to form a powder. To build the chemometrics models, pure turmeric powder was spiked with metanil yellow in the 0-50% w/w concentration range.

2.3. Analysis of Curcumin using TLC-Densitometry

The curcumin content in turmeric powder was analyzed by adding 100 mg of the powder to 1 mL of ethanol in a 2 mL microtube. Then, it was vortexed and sonicated for 60 minutes. Maceration was performed for 24 hours. Subsequently, the sample was centrifuged, and the supernatant was collected. 2 µl of the sample was applied onto a silica gel 60 F_{254} plate using a micro syringe, and a standard solution was spotted. The plate was then placed into a chamber containing a saturated mobile phase of toluene: glacial acetic acid (8:2). Elution was carried out to the boundary, followed by lifting and air-drying. Densitometric spot analysis of curcumin was performed at a wavelength of 425 nm.

2.4. FT-IR Spectral acquisition

The FTIR instrument used is the Qatar-S Single Bounce Diamond ATR (Shimadzu, Japan) with attenuated total reflectance (ATR) sample handling; the sample area is cleaned using acetone, the primary spectrum (background) is scanned before taking measurements on the sample, the sample to be checked is prepared, the sample is placed under the crystal ATR, the sample was measured in 32 scans and at a resolving power (resolution) of 16 cm^{-1}, scans were carried out at a wavelength of 4000-650 cm^{-1} and replicated two times after being recorded in the form of absorbance, after scanning, the ATR was cleaned using acetone, then dry with a tissue.

2.5. Data Processing

The data was processed using SIMCA software (Umetrics, Umeå Sweden) for OPLS-DA and then TQ Analyst software (ThermoScientific, USA) for PLS. Data (numerical and nominal data) is entered into the software, optimization is carried out, and a score plot is obtained that describes grouping OPLS-DA and R^2 in PLS.

3. RESULTS AND DISCUSSIONS

3.1. Curcumin content in pure turmeric powder

Curcumin compounds were analyzed using the TLC-Densitometry method. The average content of curcumin was found to be 3.52% (Table 1). Based on the Indonesian herbal pharmacopeia, the curcumin content in turmeric powder is about 3.82% [30]. The results obtained are slightly different from the Indonesian herbal pharmacopeia; this can be caused by some factors, such as geographical factors of the cultivating regions and the amounts of Nitrogen, Phosphorus, and Potassium in the applied fertilizers [31]. The harvest age and climate also affect the curcumin content [32].

TABLE I. Curcumin content in Turmeric powder results of TLC-Densitometry.

Sample	Sample weight (g)	Area	Curcumin (ng)	Curcumin % w/w	Average % w/w	References [30]
Turmeric powder	0.0509	17225.40	179.390	3.52	3.52	3.82
	0.0508	17135.74	178.184	3.51		

3.2 Spectral Interpretation

The spectrum of turmeric powder is shown in Figure 1(a). The spectrum and structure of metanil yellow are shown in Figure 1(b). According to both spectra, turmeric powder and metanil yellow have different profile spectra, even though they have similar colors, because they contain other compounds. Turmeric powder that contains curcumin (inset Figure 1(b)) includes six oxygen atoms, two of which are in carbonyl groups (C=O), no nitrogen or sulfur atoms, two methyl groups, and one single methylene site at the center of the molecule between the two carbonyl sites. Metanil yellow contains three nitrogen atoms (N=N and -NH) and a sulfate group (SO^{3-}) (inset Figure 1(b)). Both compounds contain substituted aromatic compounds. Turmeric powder/curcumin has two rings, and the metanil yellow has three [18].

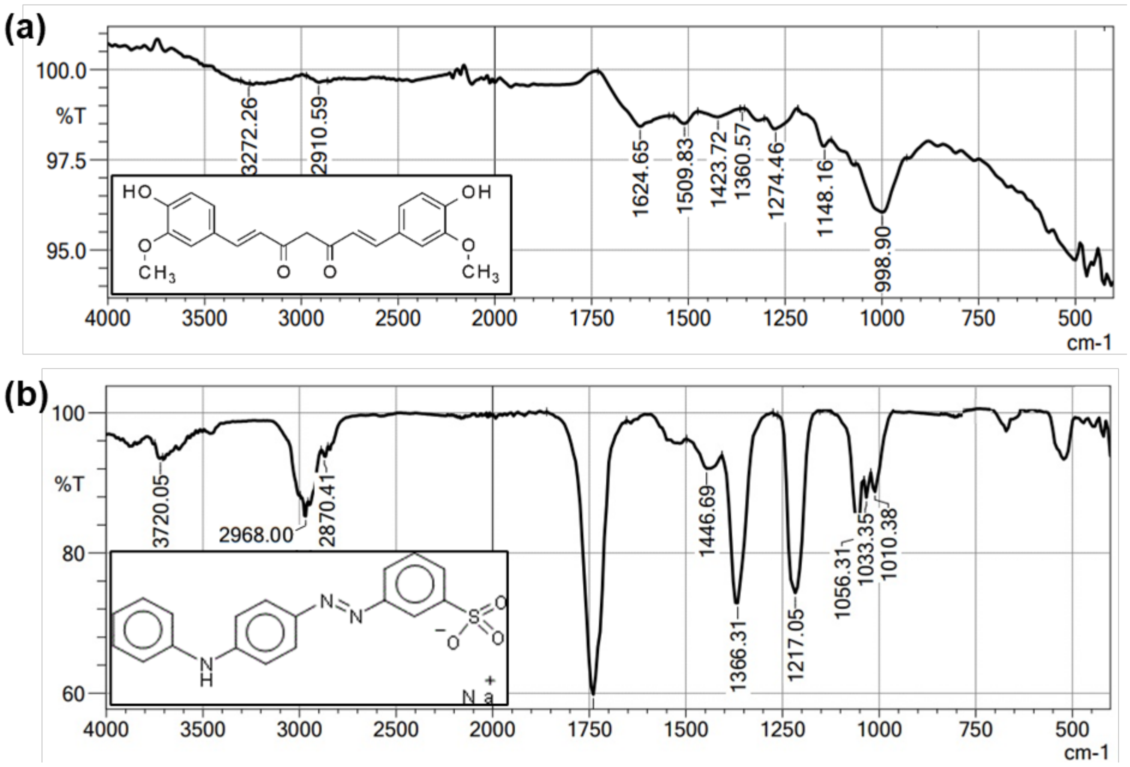

Figure 1. FTIR spectrum of (a) Turmeric powder (inset: structure of curcumin) and (b) metanil yellow (inset: structure of metanil yellow).

In turmeric powder, 3272, 2910, and 1624 cm^{-1} peaks correspond to -O-H bonding vibration, -CH_3, and -C=C- vibration. Also, some 1509, 1423, and 1360 cm^{-1} peaks correspond to -C=C- aromatic, -C-O-CH_3, and -C-H- bending vibration, respectively. While, in the spectrum of metanil yellow, there are some peaks in 2968, 1750; 1446-1366, and 1056-110 cm^{-1} corresponding to -C=O-; -S=O; C=N and N=N; and SO_3 vibration [18]. The dissimilar peaks of the functional group from the turmeric powder and metanil yellow could be used for chemometrics analysis.

3.3 Orthogonal partial least squares-discriminant analysis (OPLS-DA)

TABEL II. The validation of OPLS-DA method to classification pure Turmeric Powder and Metanil yellow.

Model	**Calibration set**	**Validation set**		**R^2X**	**R^2Y**
	Sample	Sample	Correct		
Turmeric powder	8	4	100%	0.992	0.917
Metanil Yellow	12	6	100%	0.980	0.921

After scanning each sample, the spectrums were analyzed using OPLS-DA and wavenumber 4000-400 cm^{-1}. OPLS-DA processing of turmeric powder samples and metanil yellow in Figure 2. The results showed that OPLS-DA was able to group the two samples well. Next, OPLS-DA is validated by removing group information and sample names. 1/3 of the total number of samples is used for validation, and 2/3 of the total number of samples for calibration [33]. Table II shows that OPLS-DA can group the turmeric powder and metanil yellow samples corresponding to its class with a truth level of 100%. R^2X and R^2Y for validation using turmeric powder samples are 0.992 and 0.917, respectively, and R^2X and R^2Y for validation using metanil yellow samples are 0.980 and 0.921.

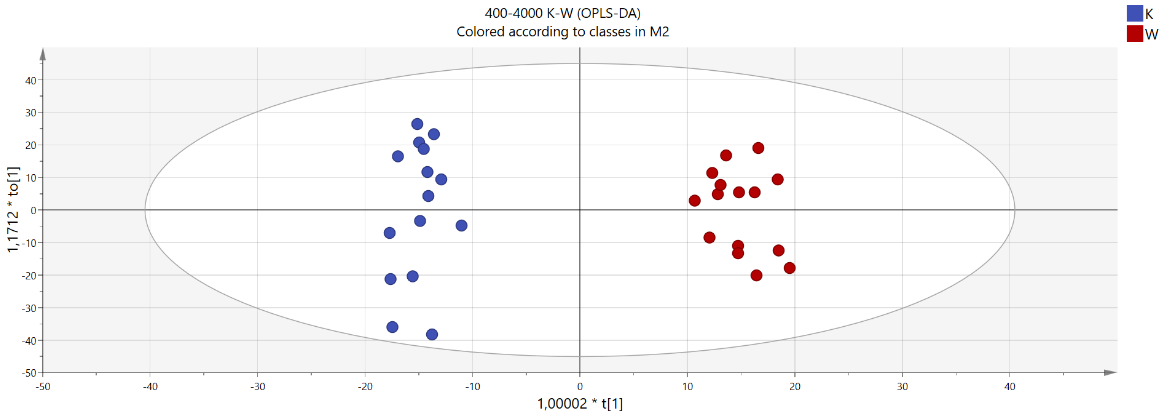

Figure 2. OPLS-DA Turmeric Powder and Metanil Yellow (K= Turmeric powder (blue colour); W: metanil yellow (red colour))

The detection of adulterants in 10 samples was carried out using OPLS-DA. Several examples of OPLS-DA results are presented in Figure 3. Based on screening using OPLS-DA, samples 1 and 2 are pure samples. This is shown by the dots of the samples 1 and 2 were in a group of pure turmeric powder. While, samples 3-10 are adulterated samples shown by the dots of the sample were in a group of turmeric powder. Next, the sample 3-10 was quantified using PLS to measure the adulterant concentration.

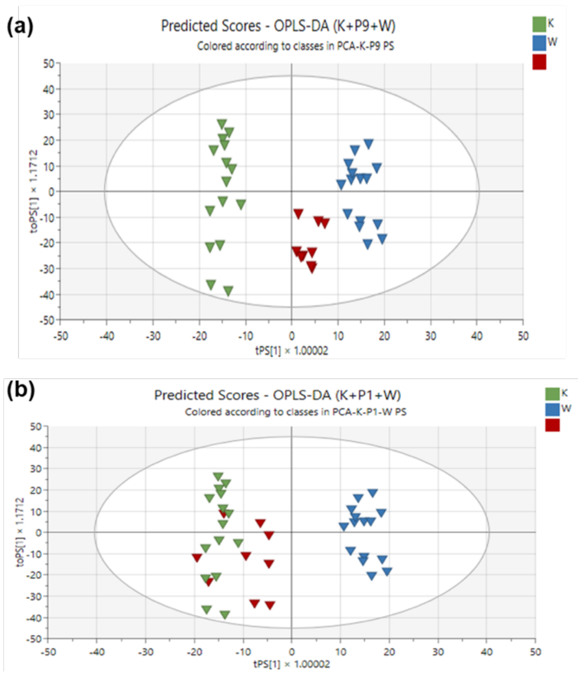

Figure 3. The several predicted scores as the results of the OPLS-DA method for detecting the adulteration in the Turmeric Powder sample (a= sample 1, pure turmeric powder; b = sample 9, adulterated turmeric powder).

3.4 Partial Least Square (PLS)

PLS was optimized to obtain the optimum wavenumber to measure the concentration of metanil yellow. The validation of the selected wavenumber is carried out using the one-leave-out validation technique. Validation is carried out on this PLS with one of the calibration samples (for example, the 10% level sample) being removed; then this sample is modeled with the remaining samples. The optimization result is shown in Table III, and the regression curve is in Figure 4. The wavenumber, which produces R^2>0.99 and the smallest RMSEC and RMSEP, was selected as the optimum condition. The chosen wavenumber value is 1199-539 cm^{-1}, where the calibration R^2 value is 0.9967; RMSEC value 2.48; R^2 calibration 0.9970; RMSEP 6.35. The samples measured using PLS were only 3-10 because the OPLS-DA results showed these adulterated samples. From the PLS result, sample 3 contains metanil yellow as an adulterant with a concentration of 9.21% (w/w), and in samples 4-10, no metanil yellow was detected, indicated by a negative value obtained. The adulterant in turmeric powder may use other ingredients.

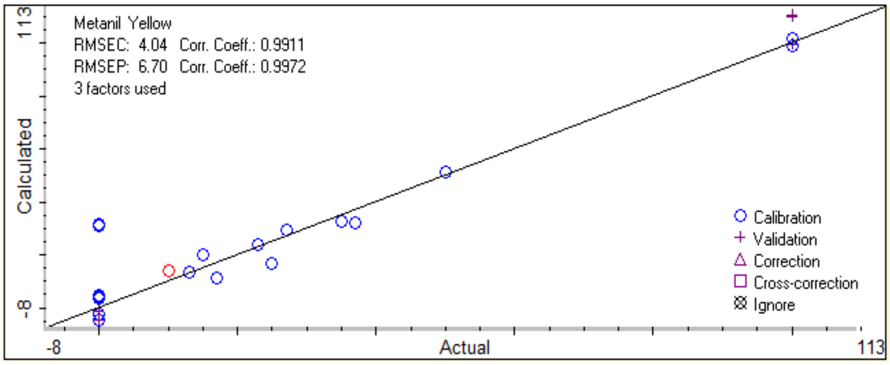

Figure 4. PLS Calibration curve turmeric powder vs metanil yellow

TABLE III. Optimization of the multivariate calibration model for quantitative analysis synthetic colour powder in turmeric powder.

Multivariate Calibration	Wavenumber (cm^{-1})	Calibration		Validation	
		R^2	RMSEC	R^2	RMSEP
PLS	4000-400	0,9852	5,21	0,9998	4,62
	3500-2000	0,2688	29,3	0,8693	54,2
	2500-600	0,9842	5,39	0,9994	3,76
	1800-1100	0,6837	22,2	0,9943	35,3
	1600-1400	0,9499	9,60	0,9931	15,5
	1199-539	0,9967	2,48	0,9970	6,35

TABLE IV. The results of Market Sample Prediction using PLS.

Sample	Metanil yellow conc. %(w/w)	Result
3	9.21± 2.22	Contain metanil yellow
4	-20.89± 0,72	This sample containing other adulterant
5	-15.81± 3,22	This sample containing other adulterant
6	-35.56± 4,10	This sample containing other adulterant
7	-15.18± 1,23	This sample containing other adulterant
8	-35.69± 0,77	This sample containing other adulterant
9	-44.53± 1,76	This sample containing other adulterant
10	-51.07± 2,59	This sample containing other adulterant

4. CONCLUSIONS

This study's FTIR spectroscopy method combined with OPLS-DA chemometrics could classify pure turmeric powder and metanil yellow with 100% accuracy. The OPLS-DA showed that samples 1 and 2 are pure turmeric powder, and the others are adulterated. Multivariate calibration using PLS produces a good calibration model with R^2 values for calibration and validation of 0.9967 and 0.9970, respectively, with RMSEC of 2.48 and RMSEP of 6.35. The PLS results showed that sample 3 contained metanil yellow, while in samples 4 to 10, no metanil yellow was detected. The ingredients added were not metanil yellow. It was concluded that FTIR combined with chemometrics could detect the metanil yellow in turmeric powder. However, the results of this method still need to be compared with other instruments to quantitatively analyze the metanil yellow content in turmeric powder.

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