

QUANTITATIVE ANALYSIS OF THE CONCENTRATION OF COUMARIN IN A BINARY MIXTURE USING TERAHERTZ SPECTROSCOPY COMBINED WITH OPTIMIZED LEAST SQUARE SUPPORT VECTOR MACHINE**

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The purpose of this study was to quantitatively analyze coumarin in a binary mixture system and to provide a more accurate and convenient potential for food safety inspection and supervision. This investigation has presented a novel terahertz time-domain spectroscopy (THz-TDS) approach and an optimized least square support vector machine (LS-SVM) to analyze the content of coumarin in the binary mixture of coumarin and vanillin. Terahertz responses of the binary mixtures have been measured and a Savitzky–Golay algorithm has been applied to process the absorption coefficient spectra. The principal component analysis has been used to extract features from the preprocessed data. The excellent prediction results can be obtained using LS-SVM optimized by sparrow search algorithm (SSA) with the coefficient of determination (R^2), root-mean-square error, and residual predictive deviation of the prediction set were more than 0.999, 0.001, and 146, respectively. The research shows that the prediction effect of the LS-SVM algorithm optimized by the SSA model is better than that of the support vector machine (SVM) algorithm and LS-SVM algorithm without the SSA model. Our research shows that the combination of THz spectrum and SSA-optimized LS-SVM is very promising for the analysis of coumarin and vanillin binary mixtures, and has great potential for the quantitative analysis of more complex multicomponent mixtures.

Keywords: terahertz time-domain spectroscopy, quantitative analysis, binary mixture, sparrow search algorithm, LS-SVM.

КОЛИЧЕСТВЕННЫЙ АНАЛИЗ КОНЦЕНТРАЦИИ КУМАРИНА В БИНАРНОЙ СМЕСИ С ИСПОЛЬЗОВАНИЕМ ТЕРАГЕРЦОВОЙ СПЕКТРОСКОПИИ В СОЧЕТАНИИ С ОПТИМИЗИРОВАННЫМ МЕТОДОМ ОПОРНЫХ ВЕКТОРОВ И НАИМЕНЬШИХ КВАДРАТОВ

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Кумарин количественно проанализирован в системе бинарных смесей для более точного и удобного контроля за безопасностью пищевых продуктов. Представлен новый подход терагерцовой спектроскопии во временной области (THz-TDS) и оптимизированного метода опорных векторов и наименьших квадратов (LS-SVM) для анализа содержания кумарина в бинарной смеси кумарина и ванилина. Измерены THz-отклики бинарных смесей, для обработки спектров коэффициента поглощения применен алгоритм Савицкого–Голея. Анализ главных компонент использован для извлечения признаков из предварительно обработанных данных. Отличные результаты прогнозирования получены с использованием метода LS-SVM, оптимизированного алгоритмом поиска воробья (SSA), с коэффициентом детерминации $R^2 > 0.999$, среднеквадратической ошибкой 0.001 и остаточным прогнозирующим отклонением 146. Показано, что прогнозирующий эффект алгоритма LS-SVM, опти-

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мизированного с помощью SSA, лучше, чем у алгоритма метода опорных векторов и алгоритма LS-SVM без модели SSA. Комбинация THz-спектра и SSA-оптимизированного метода LS-SVM перспективна для анализа бинарных смесей кумарина и ванилина, а также более сложных многокомпонентных смесей.

Ключевые слова: терагерцовая спектроскопия во временной области, количественный анализ, бинарная смесь, алгоритм поиска воробья, комбинация методов опорных векторов и наименьших квадратов.

Introduction. Vanillin is widely used in the spice industry because of its unique fragrance, which has a strong smell of milk and vanilla, and is widely used in the food, beverage, cosmetics, and pharmaceutical industries. As one of the largest spice varieties in the world, vanillin has very important economic benefits and is the only phenolic molecular compound produced industrially using biomass as a raw material. Vanillin from natural sources is widely found in many plants, including vanilla pods (2–3% of dry weight) [1]. Due to the high planting requirements of vanilla pods, the output of natural vanillin is very low, less than 1% of the vanillin is sold on the market, and the price is expensive, which is difficult for meeting market demand. Therefore, more than 90% of vanillin on the market is synthesized by a chemical method with eugenol and guaiacol as substrates. This artificial method of synthesis greatly reduces production costs, but there are problems such as high energy consumption, environmental pollution, and unnatural products [2, 3].

Coumarin(2H-1-benzopyran-2-one) is a natural ingredient in tonka bean, woodruff, and sweet clover, occurring in the essential oils of a lot of plants, especially in certain types of cinnamon [4]. Coumarin was first isolated from the tonka bean in 1822 and successfully synthesized in 1868. It smells like vanilla and is originally used as a flavoring ingredient in foods [5]. Coumarin has been suspected of genotoxicity and carcinogenicity since the 1980s. [6]. Experiments have proved that coumarin is carcinogenic to animals [7]; however, there is no clear experiments and data to prove coumarin has an obvious carcinogenic effect on human body in later stages of life [8]. However, from the perspective of safety, more and more countries prohibit or strictly control the content of coumarin in food additives. Coumarin was banned in the United States in 1954. The European Commission strictly controls the maximum content of coumarin in foods and beverages. Coumarin is also strictly prohibited in China's infant milk powder. However, in order to pursue profits, illegal businessmen still use coumarin to impersonate vanillin to improve the flavor of food [9]. Therefore, the quantitative analysis of coumarin in the binary mixture system of vanillin and coumarin is becoming more and more urgent.

At present, the detection methods of coumarin and vanillin mainly include capillary gas chromatography, gas chromatography, liquid chromatography (LC), gas chromatography-tandem mass spectrometry, capillary electrophoresis, Raman spectroscopy, voltammetry, liquid chromatography-mass spectrometry, etc. [10–16]. In 2014, Yan et al. used the liquid chromatography quadrupole linear ion trap mass spectrometry method to determine vanillin, ethyl vanillin, and coumarin in infant formula. However, these methods are tedious, time-consuming, and harmful to samples [17].

Terahertz (THz) spectroscopy is a novel method and technology. THz wave is a general term for electromagnetic waves in the ~0.1–10 THz band of the electromagnetic spectrum [18]. In this band, the corresponding wavelength range of the THz wave is ~30–3000 μm , and the wave number range is ~3.33–333 cm^{-1} . The THz wave is between microwave and infrared. Compared with other electromagnetic waves and spectrum technologies, terahertz spectrum technology has the advantages of low energy, transient, perspective, identification, and no loss, and is considered safe for biological samples [19]. At the same time, the terahertz wave measurement technology belongs to coherent measurement, which can directly measure the amplitude and phase information of the electric field, so as to obtain the relevant optical parameters of the absorption coefficient and refractive index of the sample [20, 21]. Tao Chen et al. analyzed the weak intermolecular interaction and vibration characteristics of vanillin and coumarin using terahertz spectroscopy and density functional theory [22–24]. In the same year, Tao Chen et al. compared six coumarin food additives by using terahertz spectroscopy combined with a gray wolf and SVM algorithm and identified six coumarin food additives. Nouredine Maamar et al. used THz-TDS technology to identify whether there was vanillin in textiles [25]. The above research fully shows that it is feasible to analyze coumarin and vanillin with THz-TDS. However, there is no relevant research on the quantitative analysis of coumarin concentration in the binary system of vanillin and coumarin using THz-TDS combined with machine learning.

The concentration of coumarin in the binary mixture of coumarin and vanillin has been quantitatively analyzed by THz-TDS technology combined with machine learning. The LS-SVM algorithm is optimized by

making full use of the advantages of the SSA algorithm, such as strong optimization ability and fast convergence speed. The evaluation index of the regression model shows that the reliability and prediction accuracy of the optimized regression model has been significantly improved. This indicates that the SSA optimization algorithm based on THz data has great potential for the detection of food additives and food safety.

Experimental. Coumarin and vanillin can be used without further purification (the impurity level is less than 0.05%). Vanillin and coumarin are mixed at different concentrations to form a binary mixture, as shown in Table 1. In general, in order to increase the sample thickness to avoid the standard effect and maximize the instrument bandwidth, it is often necessary to add adhesives to solid samples. In this study, polyethylene (polyethylene, sigma Aldrich, 150 mg) was used as a binder. PE has almost zero absorption to terahertz. Therefore, it was widely used in terahertz detection and quantitative analysis of solid samples. A mixture of six different coumarin and vanillin concentrations was well mixed to maintain a total weight of about 150 g. The concentration of polyethylene was continually kept at 50%. All samples were mixed repeatedly and thoroughly, and then carefully ground into powder to avoid the formation of heterogeneous clusters and aggregates in the samples, and to limit unnecessary scattering when obtaining the terahertz spectra. The powdered sample was pressed into tablets with a diameter of 13 mm under a pressure of 4 tons/cm² for about 4 minutes. The sample was prepared with an appropriate thickness, which was recorded with a digital vernier caliper. Terahertz spectral transmission was performed 10 times at different positions of each tablet sample, and spectral data were obtained for analysis.

TABLE 1. Samples Coumarin Concentration List

Sample	Coumarin concentration, % mg/100 mg	Vanillin concentration, % mg/100 mg
1	0	50
2	10	40
3	20	30
4	30	20
5	40	10
6	50	0

Terahertz time-domain spectroscopy (THz-TDS). The transmissive THz TDS system is placed in free space, as shown in Fig. 1. Considering the strong absorption effect of water molecules on THz waves, the optical path of the whole transmissive THz TDS system needs to be placed in a confined space. During the experiment, use an air dryer to fill the confined space with dry air to keep the air humidity in the confined space below 5%. The purpose of this is to exclude the influence of water molecules on the experiment. Before each sample measurement, extract a reference signal for the empty sample bin. To improve the signal-to-noise ratio and reduce the experimental error, the measured signal of each sample is measured 100 times and the average value is taken. The samples with different concentrations were measured 10 times. The program is helpful to avoid the influence of system instability on measurement and reduce the uncertainty of the experiment.

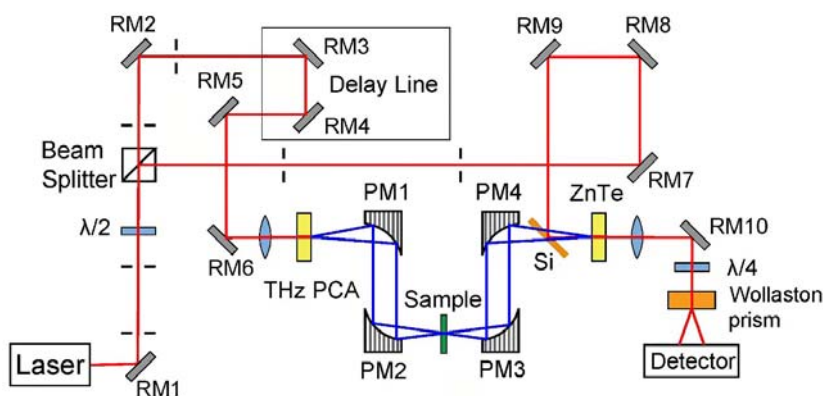


Fig. 1. Terahertz time-domain spectroscopy system.

Optical constants calculation. Optical parameters are important physical variables that can be used to describe the properties of substances. To analyze the transmission spectrum. The time-domain spectra of sample Et1 and reference Et2 were recorded respectively by measuring the sample and the empty sample bin. The data of the Et2 and Et1 are Fourier transformed into the frequency domain using fast Fourier transform (FFT) analysis by MATLAB.

After FFT transformation, the amplitude or phase information can be extracted, and then the absorption coefficient and refractive index of the sample can be calculated according to the formula. The refractive index reveals the dispersion characteristics of the sample. The absorption coefficient explains the absorption characteristics under different coumarin concentrations, which is also the basis for subsequent quantitative analysis. The specific calculation is as follows:

$$n(\omega) = \frac{c\varphi(\omega)}{\omega d} + 1, \quad (1)$$

$$\alpha(\omega) = \frac{2\omega k}{c} = \frac{2}{d} \ln \frac{4n(\omega)}{A\omega[n(\omega) + 1]^2}, \quad (2)$$

where $\alpha(\omega)$ is the absorption coefficient; $n(\omega)$ is the refractive index, in which ω is the angular frequency; d is the thickness of the tablet, c is the speed of light in a vacuum, k is the attenuation coefficient [26].

Support vector machine (SVM) is a widely used supervised learning machine learning algorithm based on a nonlinear mapping function. SVM was originally developed by Vapnik et al. [27]. SVM has two serious disadvantages. One is that the support vector machine method is powerless for large-scale training samples; secondly, solving the quadratic programming problem involves matrix inversion, which requires a lot of storage and calculation. Then, Suykens et al. put forward the concept of least squares support vector machine (LS-SVM) based on SVM, and optimized it based on the support vector machine [28]. Therefore, LS-SVM uses the square loss function to replace the insensitive loss function and converts inequality constraints such as formulas in standard SVM into equality constraints to solve the preceding two drawbacks. LS-SVM is also often used in regression analysis. Unlike SVM, the LS-SVM algorithm transforms inequality-constrained programming into equality-constrained programming based on SVM. Therefore, the solution of support SVM is transformed from a quadratic programming problem into a linear equation system, and the loss function and the sum of error squares are taken as the empirical loss of the training set. At the same time, the purpose is also changed from an optimization problem to a quadratic programming problem. Thus, the convergence accuracy and calculation speed of the LS-SVM model are improved. When constructing the LS-SVM model, we need to pay attention to two key parameters: regularization parameter C and kernel function parameter γ . In this paper, LS-SVM is used to establish a regression model with the preprocessed terahertz data as the independent variable and the coumarin concentration as the dependent variable [29].

The terahertz spectrum contains interference signals such as offset, drift, and noise, which will cover up the true terahertz response of the substance and affect the accuracy of modeling. Therefore, it is often necessary to preprocess spectral information. The preprocessing method will highlight useful information, extract more accurate and effective spectral data, improve the signal-to-noise ratio of terahertz spectral data, and reduce spectral distortion. In this article, Savitzky Golay (S-G) smoothing is used to preprocess terahertz absorption spectral data, thereby improving the stability and accuracy of quantitative detection [30].

Sparrow search algorithm (SSA) is a new swarm intelligence optimization algorithm, inspired by Sparrow's foraging behavior and evading predatory behavior and proposed by Jiankai Xue in 2020. Each sparrow represents a location attribute – that is, the location where it finds food. In the overall situation, each sparrow plays two different roles: producer, searching for food within a certain range; scrounger, following the producers to find the best food. At the same time, there are also some sparrows in the sparrow population that act as watchers. When they find predators and dangers, they will chirp. The whole flock of sparrows will fly away as anti-predatory behavior. The essence of SSA optimization is to find the position with the highest food energy step by step, that is, the optimal solution, by changing the positions of producers, scroungers, and watchers in each generation of the population. In LS-SVM, the penalty coefficient c and the kernel function parameter γ have a very important impact on the accuracy of the whole regression prediction, thus it is particularly important to select appropriate parameters [31]. This paper optimizes the parameters based on the SSA algorithm to optimize the prediction results and improve the prediction accuracy of the density. The specific process is shown in Fig. 2.

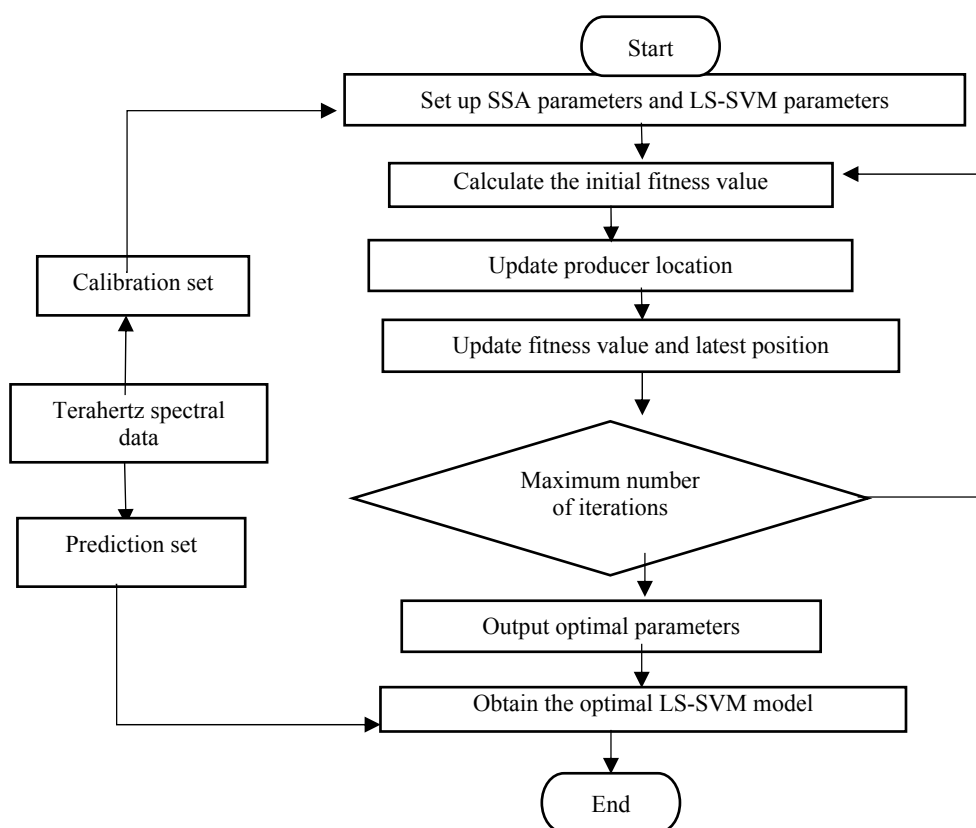


Fig. 2. SSA optimization LS-SVM flowchart.

Results and discussion. *Spectral analysis.* According to the thickness of the sample and the spectral information in the frequency domain, the absorption coefficient of the sample at different concentrations was calculated. The absorption coefficient of the binary mixture sample of coumarin and vanillin in the band of 0.25–2.0 THz band is shown in Fig. 3b. As shown in Fig. 3b, the absorption coefficient of the mixture sample varied with the concentration of coumarin and vanillin in the binary mixture. The results showed that there was an absorption peak at 1.13 THz, and the peak value of the absorption peak increased with the increase of coumarin concentration. The absorption peak of pure coumarin is at 1.15 THz, as shown in Fig. 3a. Therefore, this specific absorption peak can be used as a fingerprint feature in the quantitative analysis of coumarin and vanillin binary mixtures.

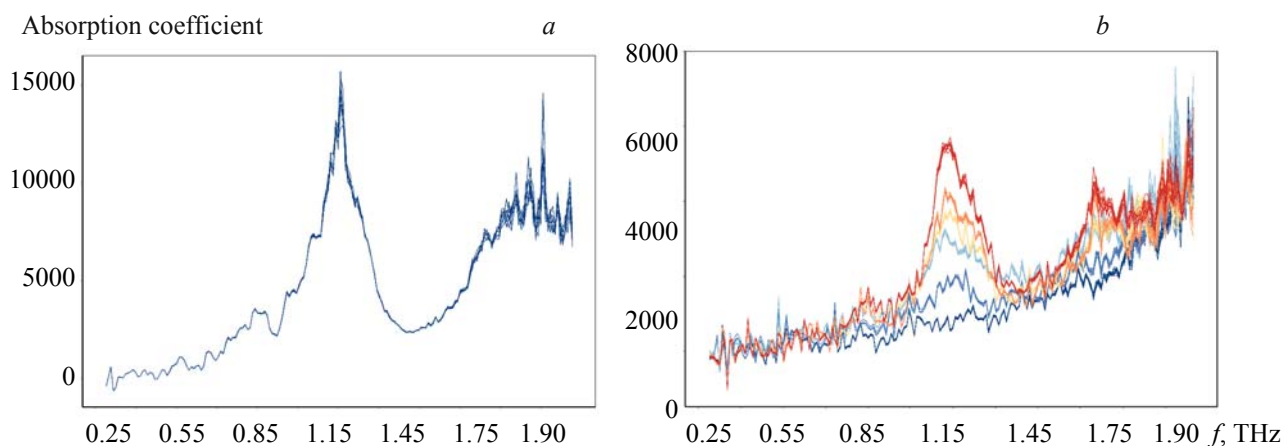


Fig. 3. Terahertz absorption spectrum of (a) pure coumarin, and (b) coumarin and vanillin binary mixtures.

Principal component analysis (PCA). The THz absorption spectrum data of six groups of samples with different coumarin concentrations in the frequency range of 0.2–2.0 THz were processed by S-G smoothing. The processed data were used as original variables for PCA analysis. Therefore, each sample data contains 164 original variables, which correspond to the absorption coefficients of different samples after pretreatment at different frequency points. PCA algorithm is used for dimension reduction and spectral feature extraction. The first six principal components PC1, PC2, PC3, PC4, PC5, and PC6 are selected as the spectral feature extraction results from the transformed results. Their contribution rates were 57.6, 18.9, 7.7, 6.5, 4.2, and 0.9%, respectively, and the cumulative contribution rate reached 95.8%. The first six principal components are generated by converting the original high-dimensional complex THz absorption spectrum data into new uncorrelated linear spectral eigenvectors, which also contain most of the THz absorption spectrum information in the 0.2–2.0 THz band. Therefore, the first six principal components can represent the original THz absorption spectrum data of samples at different coumarin concentrations while reducing the data dimension. As shown in Fig. 4, the two-dimensional score map was obtained after dimensionality reduction. After dimensionality reduction and spectral feature extraction of the data after THz absorption spectrum preprocessing by the PCA algorithm, it can be seen that samples with different coumarin concentrations can be well distinguished, especially high concentrations (50, 40, and 30%) and low concentration (30, 20, and 10%). This lays a better foundation for the subsequent establishment of regression prediction models.

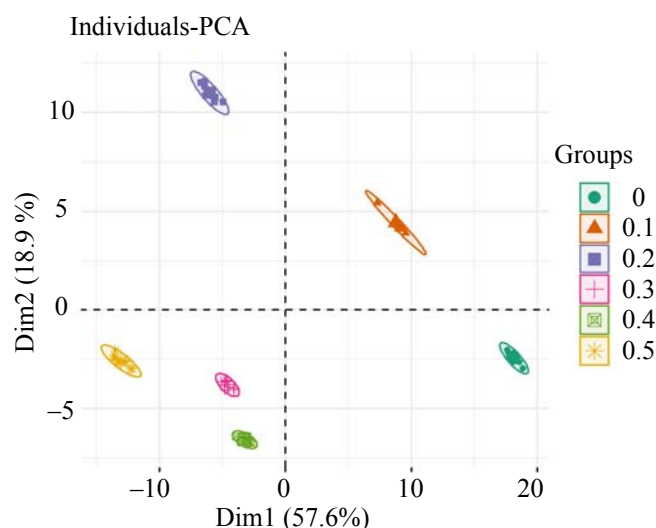


Fig. 4. The two-dimensional score map obtained after PCA.

Quantitative analysis. In addition to PE adhesive, the sample also includes vanillin and coumarin. Therefore, there are usually two methods for quantitative analysis of binary mixtures: quantitative analysis of one single analyte at a time or simultaneous quantitative analysis of two analytes. The concentration of coumarin in the binary mixture is quantitatively analyzed. The tablets of each concentration value are measured 10 times at different locations using THz. There are 6 samples with different concentrations, so, there are a total of 60 sets of original spectral data. After preprocessing (SG smoothing and PCA feature extraction), the spectral data of the academicians are randomly divided into a calibration set (80%) and a prediction set (20%). The regression model has been used for quantitative analysis of the processed spectral data.

The new method is compared with the support vector machine and the least squares support vector machine. In addition, the determination coefficient (R^2), root mean square error (RMSE), and residual prediction deviation (RPD) are used to evaluate the performance of the model. The larger R^2 , RPD, and smaller RMSE values indicate that the regression model had better prediction accuracy and stability. Figure 5 shows the predicted coumarin concentration values of SVM, LS-SVM, SSA, and SSA-LS-SVM of LS-SVM and the actual coumarin concentration values in the sample. In the Fig. 5, a red dotted line was also called the baseline (zero error), that is, $Y = X$, where Y is the coumarin concentration predicted by the model, while X represents the actual coumarin concentration of the sample. The closer the predicted value is to the baseline, the closer the predicted value is to the actual concentration value.

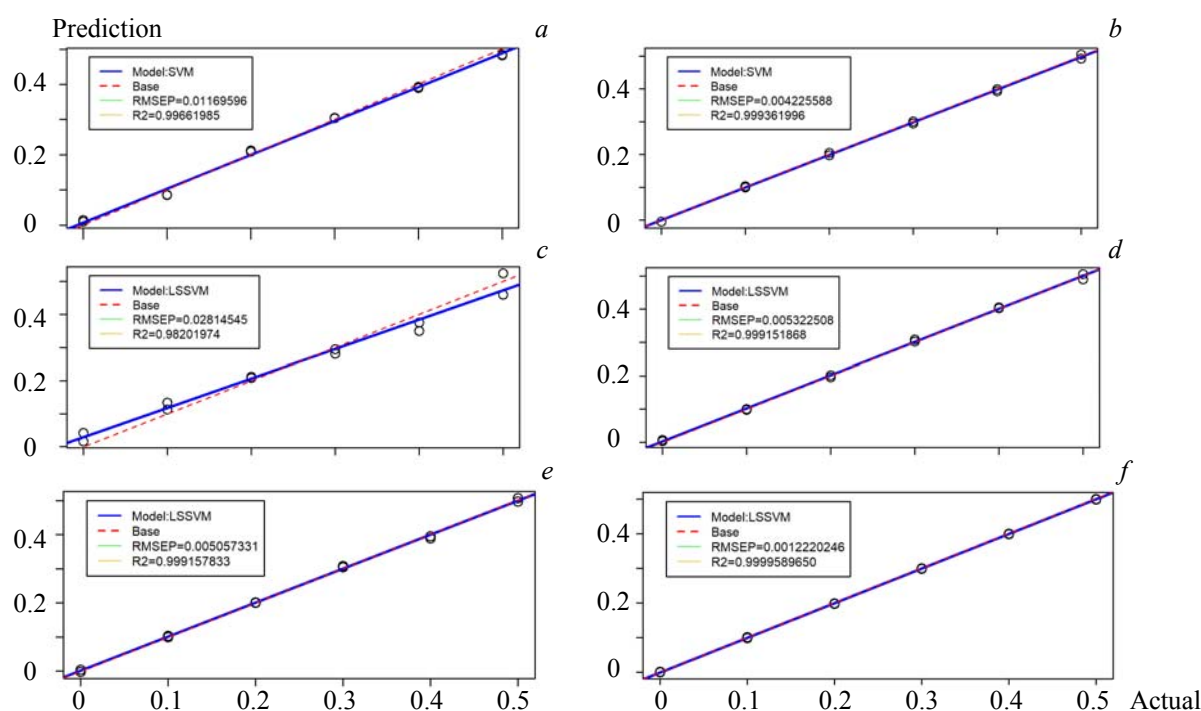


Fig. 5. SVM (a), LS-SVM (c), and SSA-LS-SVM (e) regression prediction results; SVM (b), LS-SVM (d), and SSA-LS-SVM (f) and the regression prediction results of SSA-LS-SVM (f) for terahertz spectral data PCA feature extraction. $y = x$ (the bottom of the red dotted line) is the reference line indicating the prediction reliability of each model.

In Table 2, it can be found that the prediction accuracy of the three models has been improved after PCA feature extraction. This shows that the redundant spectral data is deleted from the PCA processed data, thereby improving the performance of the regression model. Compared with other regression models, the LS-SVM model optimized by the SSA algorithm has the largest RPD and the smallest RMSE. The results show that the optimized model has better prediction accuracy and reliability. In particular, comparing the regression prediction results of the LS-SVM model before and after optimization means that the model optimized by the SSA algorithm has found the best parameters of the model, thus greatly improving the prediction performance and robustness of the model. It can also be seen intuitively in Fig. 5 that the model optimized by the SSA algorithm is closer to the baseline. R^2 , RMSE, and RPD of SVM, LS-SVM, and SAA-LS-SVM are shown in Table 2. This showed that SSA-LS-SVM has better prediction accuracy and stability than SVM and LS-SVM.

TABLE 2. Summary of Regression Analysis Results

Model	Feature extraction	Datasets	RMSE	R^2	RPD
SVM	PCA	Calibration	0.012	0.996	
		Prediction	0.012	0.997	39.675
	No	Calibration	0.001	0.999	
		Prediction	0.005	0.999	15.251
LSSVM	PCA	Calibration	0.012	0.999	
		Prediction	0.028	0.982	33.514
	No	Calibration	0.001	0.999	
		Prediction	0.005	0.999	6.338
SSA_LSSVM	PCA	Calibration	0.001	0.999	
		Prediction	0.005	0.999	145.968
	No	Calibration	0.001	0.999	
		Prediction	0.005	0.999	35.271

Conclusions. The experimental results show that SSA-LS-SVM has better prediction accuracy and stability than SVM and LS-SVM. According to the results of the regression model, the reliability and prediction accuracy of the regression model after PCA feature extraction are improved. Therefore, the SSA optimization algorithm can effectively improve the prediction accuracy of coumarin quantitative analysis. In this study, the combination of terahertz spectroscopy and the SSA optimization algorithm has shown great potential in the quantitative analysis of binary mixtures. It is of great significance in regards to eliminating the abuse of coumarin food additives.

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