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A rapid identification method of rice protein in rice starch based on synchronous fluorescence spectroscopy

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Abstract

Rice protein is a protein with high nutritional value and its essential amino acids are complete. Rice protein content is related to the Nutritional value of rice powder mixture. Three-dimensional synchronous fluorescence spectroscopy combined with Parallel factor analysis (PARAFAC) and Partial least squares (PLS) was applied for Protein content in rice starch. Synchronous Fluorescence Spectroscopy were obtained at 250–400 nm (excitation) and 260–750 nm (wave-length deviation). Firstly, data of Rice starch were collected using synchronous fluorescence spectroscopy equipment and the protein content of samples were got by Dumas Nitrogen analyzer ; secondly, three improved PLS methods, including interval partial least squares (iPLS), backward interval partial least squares (biPLS) and synergy interval partial least squares (siPLS),were used to find the most informative range. Lastly, models with better predictive turbidity of fragrance vinegar using PLS was established in the whole wavelength range .The results showed that the models by siPLS method, which separated the whole spectra into 10 intervals, and combine four intervals of [1 3 4 5]had the best predictive ability, the RMSECV and RMSEP were 0.1482 and 0.1696, and the calibration and prediction correlation coefficient were 0.8513 and 0.8236. Using three-dimensional synchronous fluorescence spectroscopy for rapid detection of Protein content in rice starch was feasible.

Keywords: rice protein; synchronous fluorescence spectroscopy; Dumas; PARAFAC; PLS.

Practical Application: These outcomes highlighted that fluorescence spectroscopy coupled with chemometrics can be used as quick method to predict the amount of rice protein. Combining the values of rice protein content at various concentration gradients determined using the Dumas combustion method with three different methods, iPLS, SiPLS, and BiPLS. The values of RMSECV and RMSEP were similar, and the correlation coefficients of the calibration set and prediction set were also similar. It shows that the accuracy of actual application is the same as that of prediction set and the practical application is feasible. It indicate that fluorescence spectroscopy can be used in the rice protein industry as a problem-solving tool and as a rapid and comprehensive approach for on-the-spot detection of rice protein quality.

1 Introduction

Rice is one of China's most important food crops (Li & Li, 2020), Rice contains 6-8% of the protein (Yao et al., 2022). With the advancement people are gradually becoming aware of rice protein's high nutritional value, and it has become a major trend to develop it as a processing base for food or nutrition and health products. (Koo & Lasekan, 2007). Rice contains four proteins: albumin, globulin, prolamin, and glutelin, the latter of which contains the most gluten (Chen et al., 2018).Since rice protein contains lysine with high-quality amino acid composition, it is hypoallergenic and, therefore, favorable for human consumption (Roa-Acosta et al., 2022). Food safety is one of the major challenges of countries in underdeveloped countries and developed and developing countries (Mahmudiono et al., 2022). With the extensive research on rice protein, there is an urgent need to develop a rapid detection method for rice protein. There are many methods for protein detection, the most commonly used are Kjeldahl method, Lowry method, Dumas method and Bradford method (Bonjoch & Tamayo, 2001; Jiang et al., 2013). It is recommended the use of combustion methods for their lower production of environment- and health-hazardous residues and the rapid analysis (Cenci et al., 2021) .However, the procedure is tedious, the detection takes an extended period of time, and also requires the consumption of large volumes of reagents. Beyond that, Tedious operation also cause damage to the product during the detection (Zhongfu et al., 2015).

Because of advances in spectroscopy and the development of novel sensor technologies such as spectrophotometers and cameras, non-destructive and rapid assessments of quality-related factors in foods are becoming more common (Pranoto et al., 2022). In the past few decades, a good number of rapid nondestructive detection technologies have been developed for the safety and quality detection of various agricultural products, one of which is the Synchronous fluorescence spectrum (Xin et al., 2022; Mahmudiono et al., 2022). Synchronous fluorescence spectroscopy is a change at the same time under the condition of excitation wavelength and emission wavelength scanning some with fluorescent effect of substances such as amino acid,

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measured by the fluorescence intensity of signal is the method of qualitative and quantitative analysis. (Muhammad et al., 2019). It is a non-destructive and rapid detection technology that generates composite information that distinguishes one sample from another. This technology is capable of rapidly analyzing a large number of samples. It is highly automated and has significant development potential. Synchronous fluorescence spectra produces no pollution and boasts high sensitivity and strong selectivity (M. Chen et al., 2014). Synchronous fluorescence spectrometry in food of rapid detection and identification of applied research is developing rapidly (Karoui & Blecker, 2011). Such as based on synchronous fluorescence spectroscopy combined with chemometrics analysis applied in eucommia ulmoides seed oil adulteration detection (Ke-Qing et al., 2019). Synchronous fluorescence detection of oil and olive oil adulteration identification (Fang et al., 2015). Fluorescence spectroscopy is also reported to be used for monitoring the structural changes during cheese manufacturing (Muhammad et al., 2019). Employing artificial neural networks and fluorescence spectrum for food vegetable oils identification (Pranoto et al., 2022). Rapid identification of sunflower seed oil quality by three-dimensional synchronous fluorescence spectrometry (Shuang-Fang et al., 2018). Fluorescence spectroscopy has been utilised to characterise ghee extracted from buffalo and cow milk. The spectral differences were highlighted through principle component analysis that has been applied for the detection of adulteration of cow milk in buffalo ghee (Ahmad & Saleem, 2020). EDXRF has potential to be used as a rapid offline analytical technology suitable for the analysis of these minerals in skim milk powders (McCarthy et al., 2020). The heat-acid-induced coagulation behaviour of whole milk system (buffalo, cow and mixed milk)was studied by steady-state fluorescence spectroscopy exploiting tryptophan as a marker molecule (Chakraborty et al., 2020).

At present, there are many methods for protein detection, the most commonly used are Kjeldahl method, Coomassie brilliant blue method. Although there are many ways to detect protein, there are few methods that can be used to detect rice protein. Although Kjeldahl nitrogen method is the international standard test method for the detection of protein content, it can adapt to a wide range of samples, has good accuracy, and relatively few interference factors. It can measure the absolute concentration of protein, and is often used for the accurate determination of standard protein content. However, the operation is tedious, the detection consumption time is relatively long, the consumption of reagents is also large, and the sensitivity is low (Wu, 2012). If starch contains a large amount of protein, the yield of rice protein will be reduced, and the rice protein can not be fully used effectively. However, if the purity of rice protein is not high, the large amount of rice starch will affect the quality of the product. Therefore, it is of great significance to detect the content of rice protein in rice starch.

In this study, the fluorescence spectra of rice protein in different proportions were obtained by synchronous fluorescence spectrometry, and the content of rice protein in different concentration gradients was determined by Dumas combustion method. PARAFAC was used to simplify the three-dimensional synchronous fluorescence spectrum into two-dimensional data, and the regression model was established by combining iPLS, SiPLS and BiPLS. Various pattern recognition methods were used to analyze the fluorescence spectra in order to quantify the protein content of the mixture powder based on the fingerprinting pattern of protein substances.

2 Materials and methods

2.1 Samples preparation

Rice protein and starch were purchased from Anhui Shunxin Shengyuan Biological Food Co., Ltd. A certain amount of rice protein powder and rice starch powder were weighed by balance to prepare rice starch samples with a certain percentage of rice protein. Gradient samples were prepared: Rice protein content was prepared from 5% to 100% at a gradient of 2.5%. Each gradient was configured with three parallel samples, a total of 117 samples were prepared.

2.2 Instrument and equipment

Cary Eclipse Fluorescence Spectrophotometer: Warian Co., Ltd. USA; Dumas Protein Analyser: Alimonta Trading (Shanghai) Co., Ltd; SQP Analytical Balance (d=0.01mg), Sedoris Scientific Instruments (Beijing) Co., Ltd.

2.3 Determination of the Nitrogen Content of the powder samples by the Combustion (Dumas) Method

Determination of the amount of nitrogen in the rice powder samples were done in accordance with method (Miller et al., 2007) . The detail steps were carried out as follows: Thirty-nine samples with different concentration gradient of rice protein were packed in nitrogen-free tin foil, pressed, extruded and placed in an automatic sampler. The samples were then burned under a flow of pure oxygen in a combustion tube 300s. Interfering gases generated during the combustion process were reduced using high temperature copper powder, then adsorbed and dehydrated using porous ceramics. The gases were detected using a TCD detector and their concentrations were calculated and quantified using standard curve. The reaction conditions= included a combustion tube temperature of 990 °C, and a reduction tube temperature of 650 °C. The combustion was carried out completely at 170 ml/min for 300 s until the oxygen residual reached 12%. At the same time, the mass gradient of purified reagent grade ethylene diamine tetraacetic acid (EDTA) was determined and a standard curve was drawn.

2.4 Synchronous Fluorescence spectra acquisition

The prepared samples were detected using the built-in optical fiber of the Cary Eclipse Fluorescence Spectrophotometer. Three-dimensional synchronous fluorescence spectroscopy was performed under the following conditions: The scanning range for excitation wavelength (Ex) was 200-700 nm, the wavelength difference ($\Delta\lambda$) was 10-180 nm, the excitation slit was 5 nm, the emission slit was 5 nm, the scanning speed was fast, and the interval was 2 nm (Lei et al., 2018). Each sample was scanned three times, and the average of the three scans was used as the spectral data.

2.5 Data processing method

In this study, two pattern recognition methods were performed for data analysis. PARAFAC was used to reduce three-dimensional synchronous fluorescence spectroscopy to two dimensional data (Barreto et al., 2021). PLS were used to build regression models between the synchronous fluorescence and Protein Content (Huan et al., 2014). Synchronous fluorescence spectroscopy is composed of three-dimensional data, with fluorescence intensity as the first mode, excitation wavelength as the second mode, and Δλ as the third mode. Suitable PARAFAC components are important for decomposing the three-dimensional data (Stedmon et al., 2003; Stedmon & Bro, 2008). Then, a loading score corresponding to the con- centration of the fluorescence intensity is used to select a suit- able Δλ to decompose the three-dimensional matrix into two-dimensional data.In the simultaneous fluorescence analysis, the selection of Δλ value is more important, and it has a great influence on the sensitivity and peak separation of the system. Therefore, it is very important to choose the Δλ value suitable for the mixed components. After PARAFAC Analysis, Two-dimensional data from the synchronous fluorescence spectroscopy was selected (Barreto et al., 2021).PLS algorithm the spectral decomposition and return at the same time, the concentration of matrix information is introduced into the spectral matrix decomposition process, which can maximize the use of the linear relationship between the spectral data with principal component concentration (Lei et al., 2021). Both implementations of the PARAFAC and PLS methods were performed using Matlab R2010a (MathWorks, Inc.,Natick,MA, USA).

3 Results and discussion

Three-dimensional fluorescence spectra for each commercial rice protein were calculated and analysed using the PARAFAC method to convert three-dimensional data to two-dimensional data. Then, using the PLS method (Wei & Wei, 2013), a model for detecting the protein content in rice starch was established.

3.1 Dumas result

In Table 1, the purity of the rice protein obtained from the company was 78.36%, and the error value between the dispensing concentration and the actual concentration increased slowly as the dispensing concentration was increased. This is because rice protein is easily adhered to surfaces, and as the amount of rice protein weighed increases, the amount of sticky on the top of the weighing paper is relatively more. Additionally, there is a loss in the process of allowing the rice protein and rice starch mix. However, the linear relationship between the concentration of the sample and the actual concentration showed that R^2=0.9989 in Figure 1, which indicated that the detection results were accurate enough and the Dumas combustion method was suitable for the detection of protein in rice starch.

3.2 Three-dimensional fluorescence spectra of Gradient samples

Three-dimensional simultaneous fluorescence spectrogram is not only informative, but also efficient in detecting substances because it is a combination of information from three aspects: Emission wavelength, Excitation wavelength and fluorescence intensity (Lei et al., 2018).

Table 1. Results of rice protein assay with different concentration gradients.

Sample concentration (%)	N (%)	Protein Factor	Measured protein concentration (%)
100.0	13.17	5.95	78.36
99.5	12.98	5.95	77.23
98.0	12.82	5.95	76.26
96.5	12.59	5.95	74.88
95.0	12.35	5.95	73.46
93.5	12.25	5.95	72.91
92.0	11.99	5.95	71.36
90.5	11.84	5.95	70.44
89.0	11.60	5.95	69.00
87.5	11.41	5.95	67.88
86.0	11.28	5.95	67.09
84.5	11.02	5.95	65.57
83.0	10.84	5.95	64.51
81.5	10.72	5.95	63.78
80.0	10.46	5.95	62.21
78.5	10.31	5.95	61.31
77.0	10.10	5.95	60.11
75.5	9.90	5.95	58.89
74.0	9.65	5.95	57.4
72.5	9.48	5.95	56.42
71.0	9.22	5.95	54.87
69.5	9.19	5.95	54.66
68.0	9.02	5.95	53.68
66.5	8.78	5.95	52.25
65.0	8.48	5.95	50.46
63.5	8.49	5.95	50.5
62.0	8.28	5.95	49.25
60.5	7.94	5.95	47.24
59.0	7.90	5.95	47.01
57.5	7.81	5.95	46.44
56.0	7.51	5.95	44.70
54.5	7.35	5.95	43.73
53.0	7.13	5.95	42.42
51.5	6.90	5.95	41.04
50.0	6.76	5.95	40.20
48.5	6.71	5.95	39.95
47.0	6.44	5.95	38.30
45.5	6.31	5.95	37.53
44.0	6.06	5.95	36.06
42.5	5.84	5.95	34.72
41.0	5.56	5.95	33.08
39.5	5.42	5.95	32.26
38.0	5.25	5.95	31.22
36.5	5.08	5.95	30.23
35.0	4.89	5.95	29.10
33.5	4.61	5.95	27.43
32.0	4.58	5.95	27.22
30.5	4.44	5.95	26.41
29.0	4.14	5.95	24.64
27.5	4.12	5.95	24.48
26.0	3.78	5.95	22.49
24.5	3.82	5.95	22.73
23.0	3.44	5.95	20.48
21.5	3.29	5.95	19.60
20.0	3.19	5.95	18.96
18.5	3.18	5.95	17.97
17.0	2.78	5.95	16.57
15.5	2.47	5.95	14.72
14.0	2.20	5.95	13.09
12.5	1.76	5.95	10.49
11.0	1.69	5.95	10.05
9.5	1.45	5.95	8.62
8.0	1.21	5.95	7.21
6.5	1.07	5.95	6.35
5.0	0.81	5.95	4.79

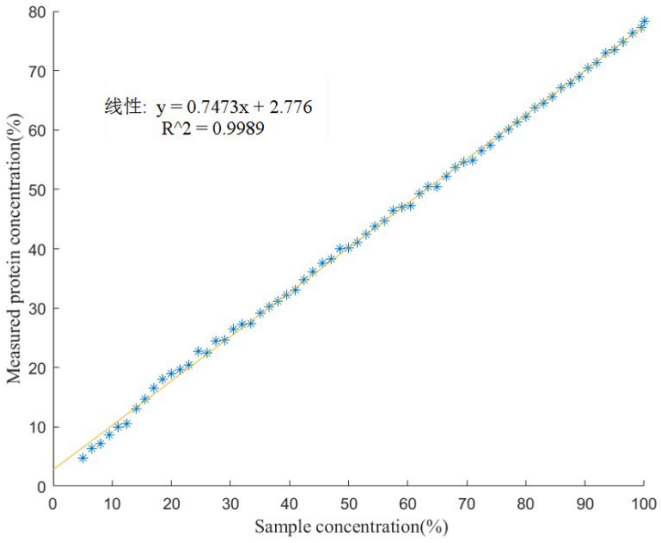

Figure 1. The linear relationship between dosing concentration and actual concentration.

It can be seen from the diagram of rice protein at different concentration gradients in Figure 2, there are obvious characteristic peaks at Δλ=30 nm and excitation wavelengths of 400 nm~600 nm for different concentrations of rice protein, which are mainly caused by protein, fat and vitamin combined, and the excitation wavelengths of the characteristic peaks become longer as the protein concentration increases, indicating that rice protein plays a major role. Therefore, the three-dimensional fluorescence spectrogram can be used to determine the rice protein concentration.

3.3 Screening characteristic wavelength Δλ by PARAFAC Analysis

It is well established that when the sum of squared residuals is smaller and the sum of squared residuals does not change significantly as a result of changing the factors, the smaller number of factors is chosen. The number of factors selected to construct a parallel factor model is more accurate when this principle is followed. The residual sum of squares of the model decreases

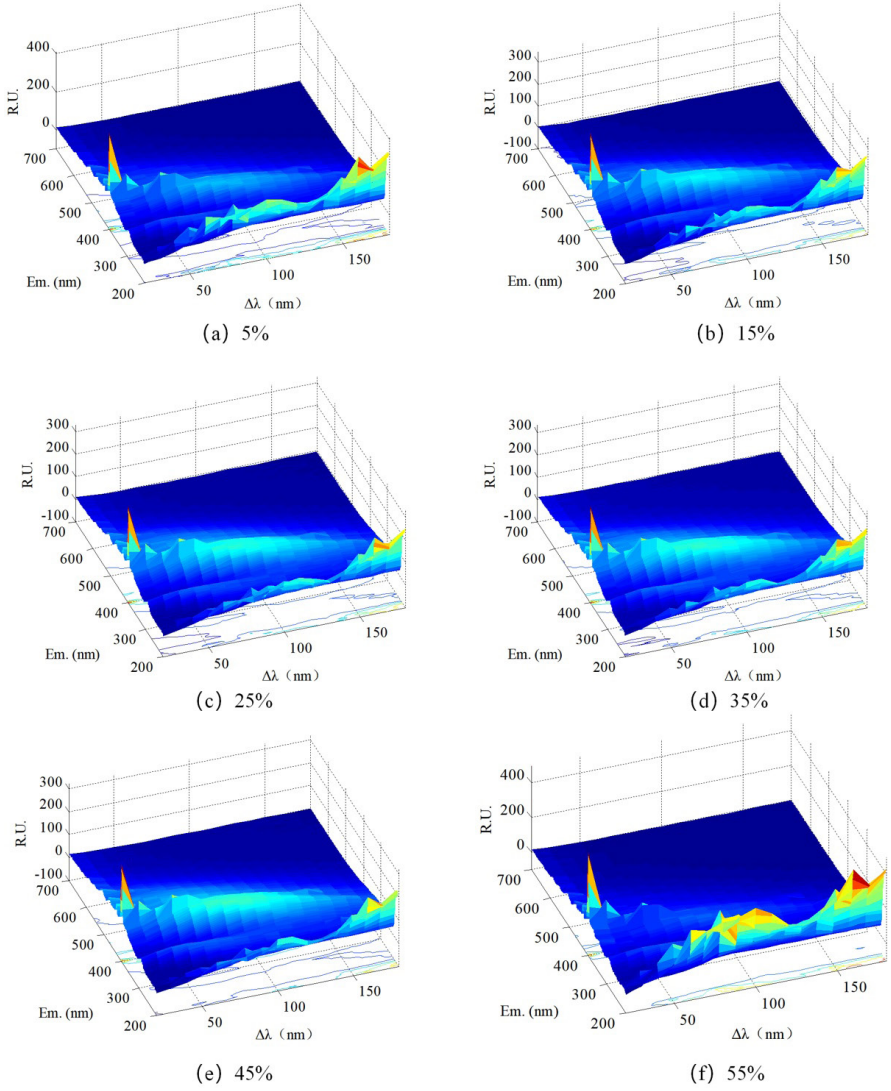

Figure 2. The Three-dimensional fluorescence spectra of Rice Starch and protein with different concentration gradients [(a) 5%, (b) 15%, (c) 25%, (d) 35%, (e) 45%, (f) 55%)].

as the number of factors increases, although the residual sum of squares of factor 9 is the smallest, making modelling time consuming. On balance, the parallel factor model is constructed using a factor number of eight in Figure 3.

If the Δλ loading value is higher, it indicates that the variability between samples is greater corresponding to Δλ, implying that the differentiation effect is also better. Because the loading value was largest at Δλ=30 nm, which corresponds to rice protein, Δλ=30 nm was selected as the characteristic fluorescence spectral data of rice protein in Figure 4.

In Figure 5, there was a distinct absorption peak near the emission wavelength of 280 nm, which was caused by tryptophan, tyrosine and phenylalanine in the protein (Karoui & Blecker, 2011). Additionally, the overall trend of fluorescence intensity increased as the concentration of samples increased.

3.4 Regression results by Different PLS models

These outcomes highlighted that fluorescence spectroscopy coupled with chemometrics can be used as quick method to predict the amount of rice protein. Combining the values of rice protein content at various concentration gradients determined using the Dumas combustion method with three different methods, iPLS, SiPLS, and BiPLS. The values of RMSECV and RMSEP were similar, and the correlation coefficients of the calibration set and prediction set were also similar. It shows that the accuracy of actual application is the same as that of prediction set and the practical application is feasible. It indicate that fluorescence spectroscopy can be used in the rice protein industry as a problem-solving tool and as a rapid and comprehensive approach for on-the-spot detection of rice protein quality.

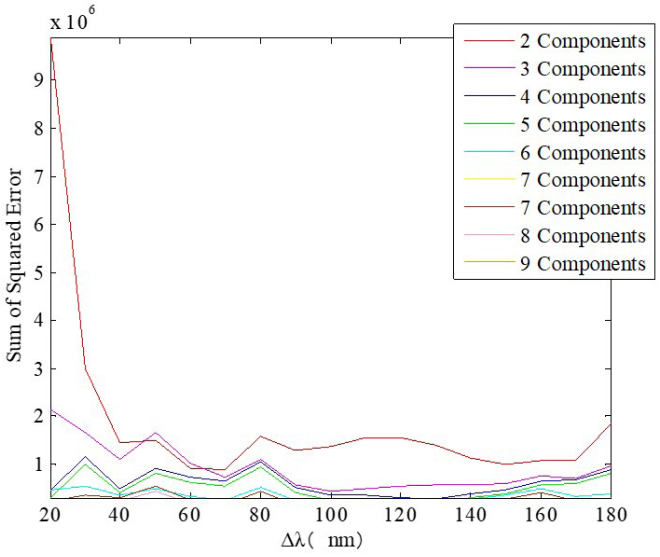

Figure 3. The Comparision of sum of squared error in different factor numbers.

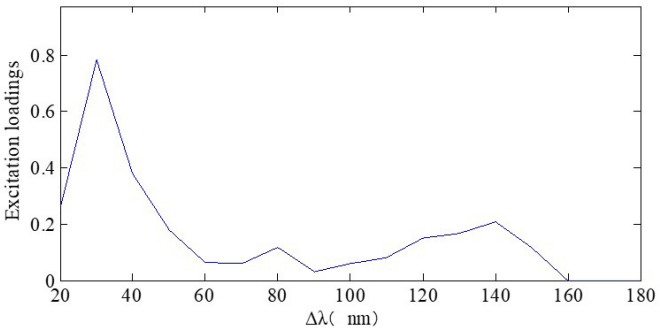

Figure 4. Δλ loadings.

The most informative ranges of PLS

The spectra were divided into one equal-width intervals, and four equal-width intervals were combined to obtain the best principal factor number of two in Figure 6. The RMSECV and RMSEP values were 0.1634 and 0.167, and the correlation coefficients of the correction and prediction sets were 0.8171 and 0.8130 in Figure 7.

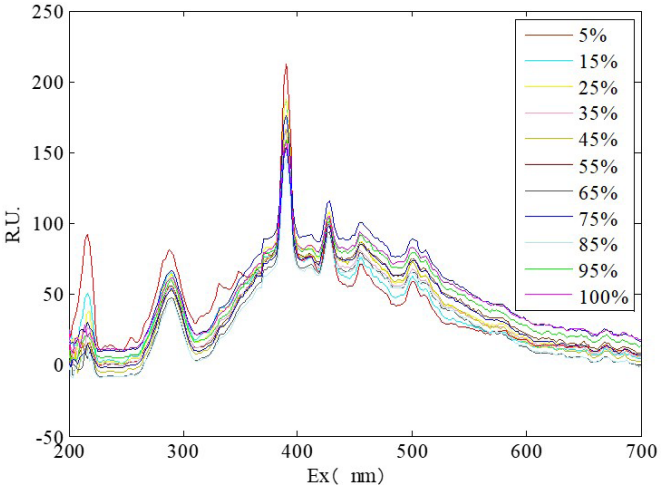

Figure 5. The two synchronous fluorescence spectra of Rice Starch and protein with different mixing ratio.

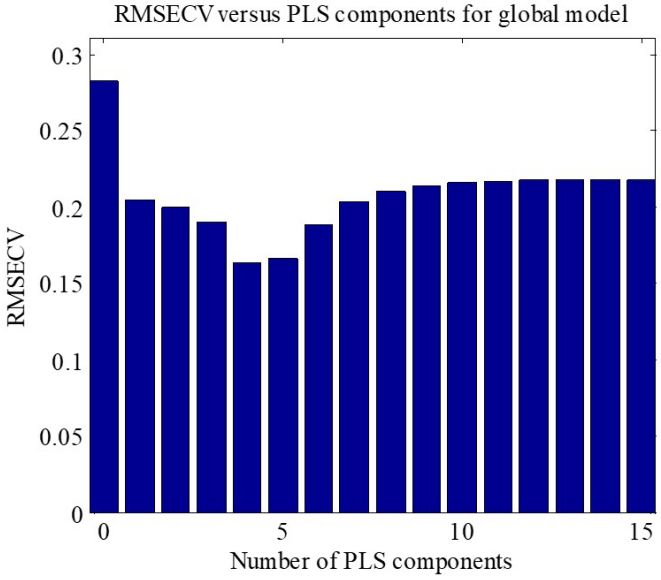

Figure 6. Master factor number of PLS model and RMSECV correlation plot.

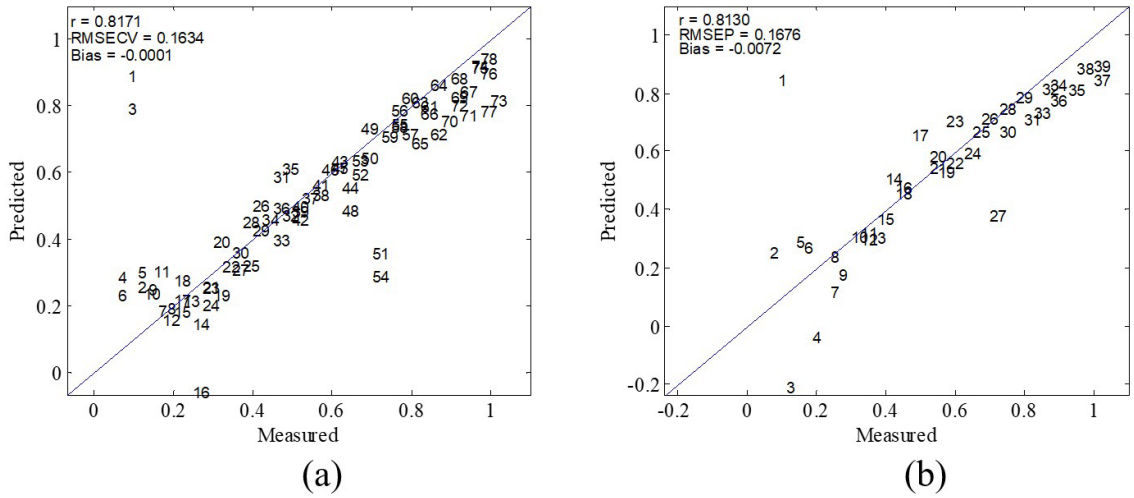

Figure 7. Correlation coefficient of calibration (a) and prediction (b) for iPLS optimization.

The most informative ranges of iPLS

The spectra were divided into 10 subintervals using the values of R, RMSECV, and RMSEP in Figure 8, and the fifth subinterval with the best principal factor number of 2 was selected in Figure 9. The RMSECV and RMSEP were 0.1926 and 0.1955, and the correlation coefficients of calibration set and prediction set were 0.7325 and 0.7141, respectively, corresponding to the wavelength range of 394~428 nm in Figure 10.

The most informative ranges of pls SiPLS

The spectra were divided into 10 equal-width intervals in Figure 11, and four of the intervals were combined to obtain the best result at a principal factor of two in Figure 12. The RMSECV and RMSEP values were 0.1482 and 0.1695, respectively, and the correlation coefficients between the correction and prediction sets were 0.8513 and 0.8236, respectively, corresponding to the wavelength ranges of 250~285 nm, 320~355 nm, 355~390 nm, 390~ 425 nm in Figure 13.

The most informative ranges of pls BiPLS

The spectra were divided into 10 equal-width intervals in Figure 14, and three with the optimal number of principal factors of two were combined in Figure 15. The RMSECV and RMSEP values were 0.1512 and 0.1677, respectively, and the correlation coefficients of the calibration set and prediction set were 0.8445 and 0.8213 in Figure 16, respectively, corresponding to the wavelength ranges of 250~285 nm, 400~435 nm, and 435~470 nm.

Comparison of prediction results from different PLS models building

Different PLS regression models established by varying the number of demarcation intervals, the number of joint intervals, the interval selection, and other parameters. SIPLS (joint four intervals) produces the best results because it divides several intervals more precisely into the same subintervals in which the local models are combined and then the prediction models are constructed. The results of models constructed using various PLS are shown in Table 2.

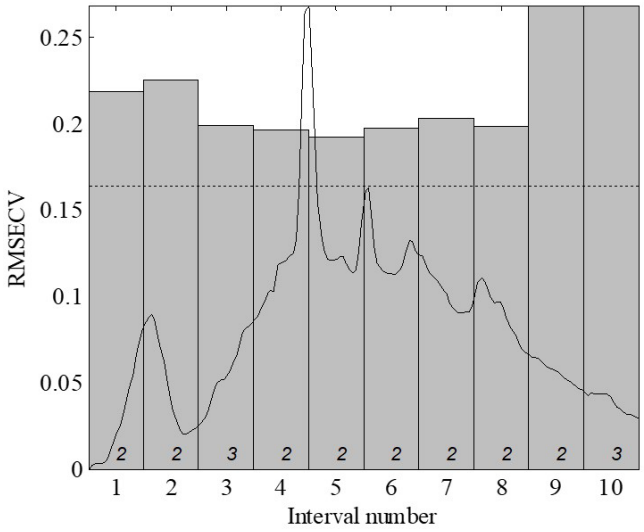

Figure 8. Optimal interval and optimal factor obtained by iPLS.

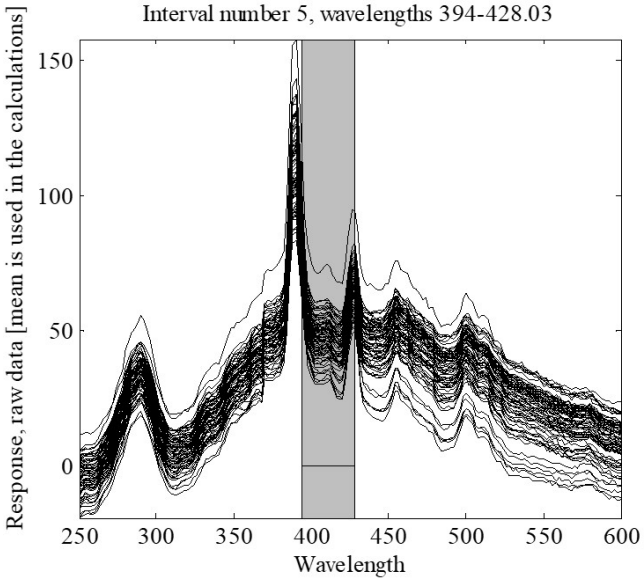

Figure 9. The wavelength range corresponding to the interval.

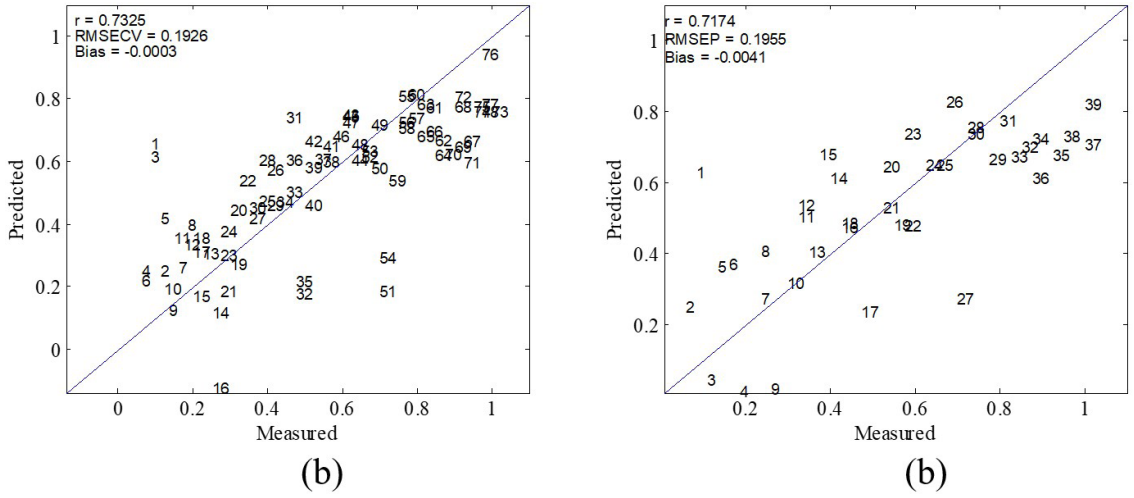

Figure 10. Correlation coefficient of calibration (a) and prediction (b) for iPLS optimization.

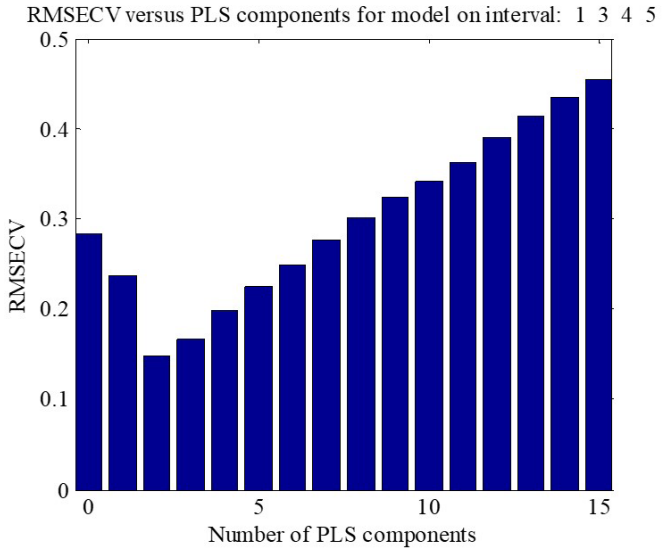

Figure 11. Master factor number of SiPLS model and RMSECV correlation plot.

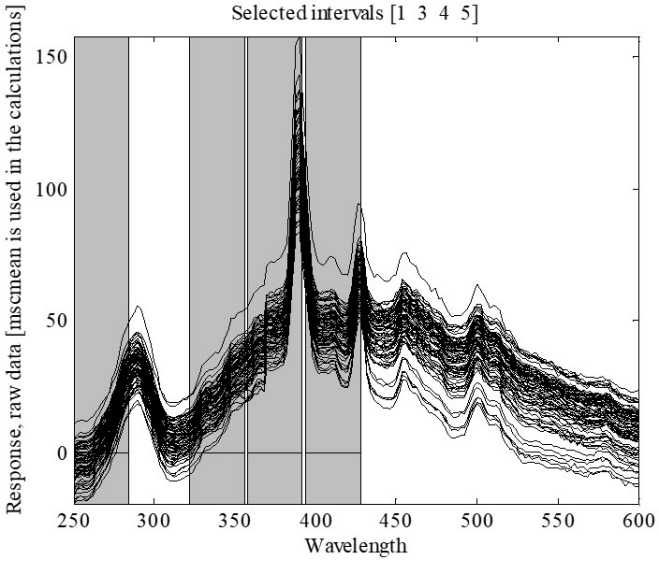

Figure 12. The wavelength range corresponding to the interval.

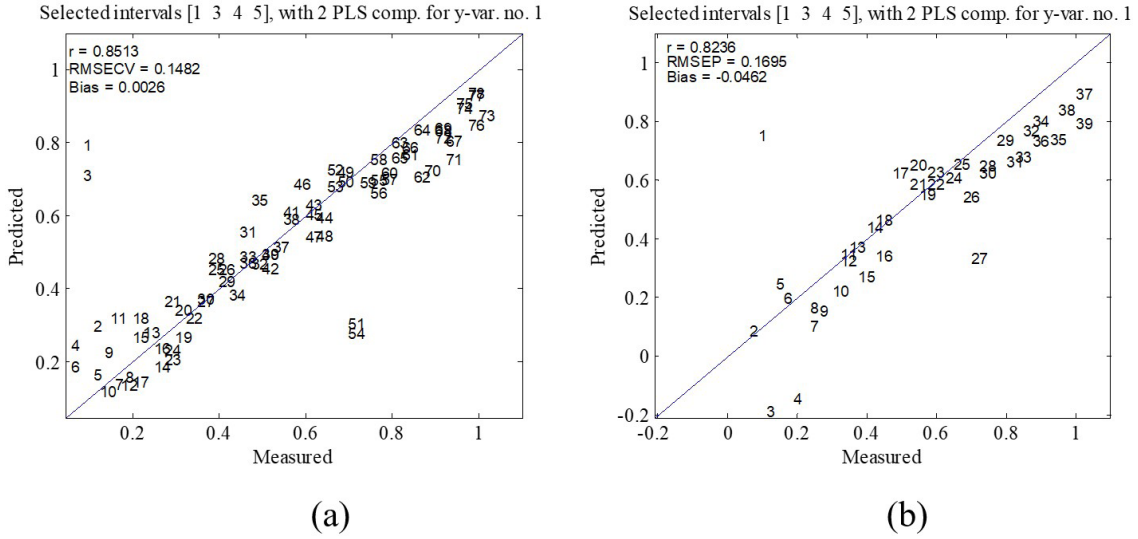

Figure 13. Correlation coefficient of calibration (a) and prediction (b) for iPLS optimization.

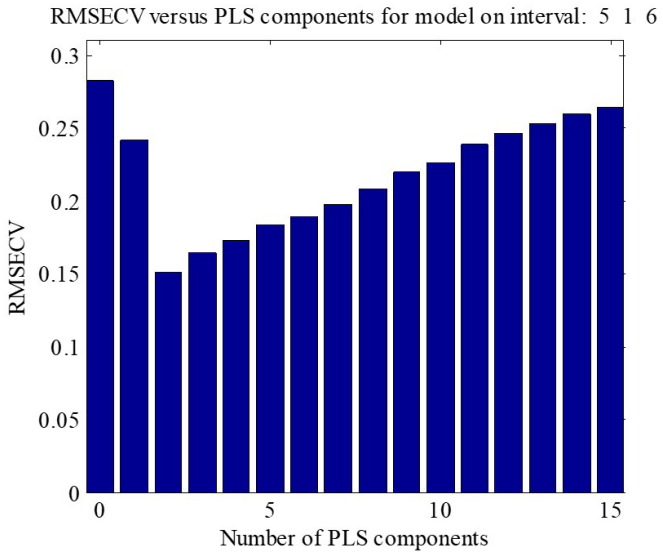

Figure 14. Master factor number of BiPLS model and RMSECV correlation plot.

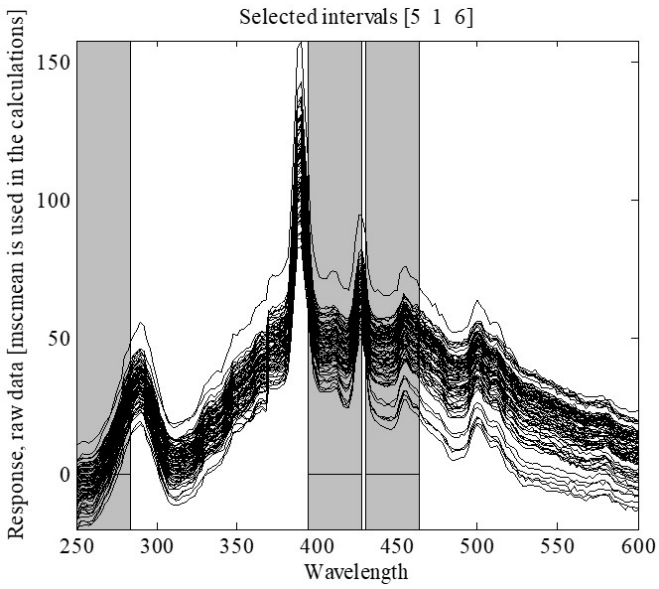

Figure 15. The wavelength range corresponding to the interval.

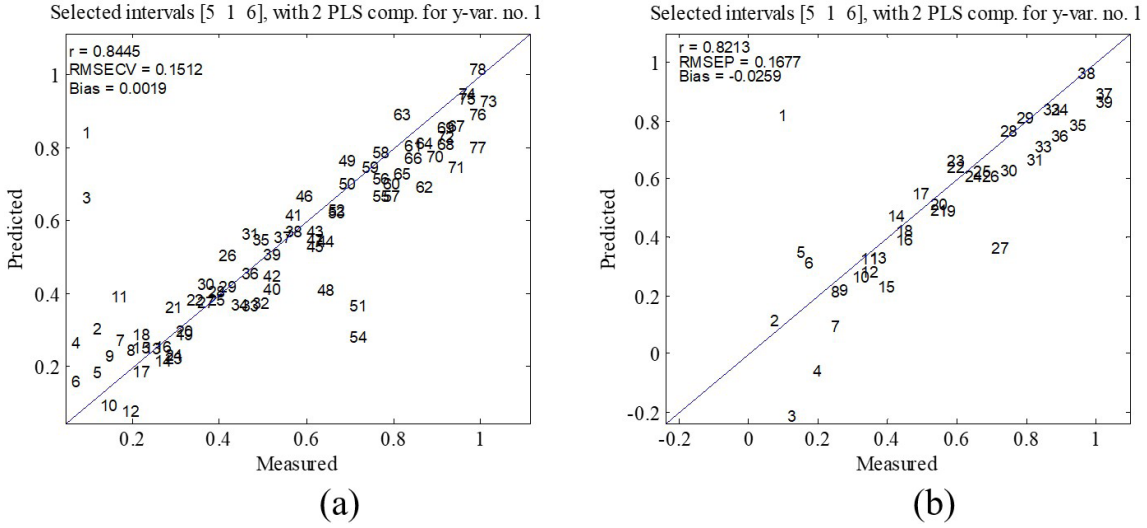

Figure 16. Correlation coefficient of calibration (a) and prediction (b) for iPLS optimization.

Table 2. Results of calibration models built by different PLS.

Modeling Methods	Number of delineated intervals	Select Interval	Optimal number of main factors	Correction set		Prediction set	
				R	RMSECV	R	RMSEP
PLS	1	Full Spectrum	2	0.8171	0.1634	0.8130	0.1676
iPLS	10	5	2	0.7325	0.1926	0.7174	0.1955
SiPLS	10	[1 3 4 5]	2	0.8513	0.1482	0.8236	0.1696
BiPLS	10	[5 1 6]	2	0.8445	0.1512	0.8213	0.1677

4 Conclusion

PLS with $\Delta\lambda$ = 30 nm was used to screen for the best characteristic of rice protein. Combining the values of rice protein content at various concentration gradients determined using the Dumas combustion method with three different methods, iPLS, SiPLS, and BiPLS, a model for the detection of protein in rice starch was established, and the model's cross-validated root mean square error and correlation coefficient were used as model evaluation indices. The results indicated that the SiPLS method combined interval [1 3 4 5] was the most accurately modelled, with RMSECV and RMSEP values of 0.1482 and 0.1696, respectively. Thus, three-dimensional synchronous fluorescence provides a theoretical foundation for rapidly determining the protein content of rice.

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