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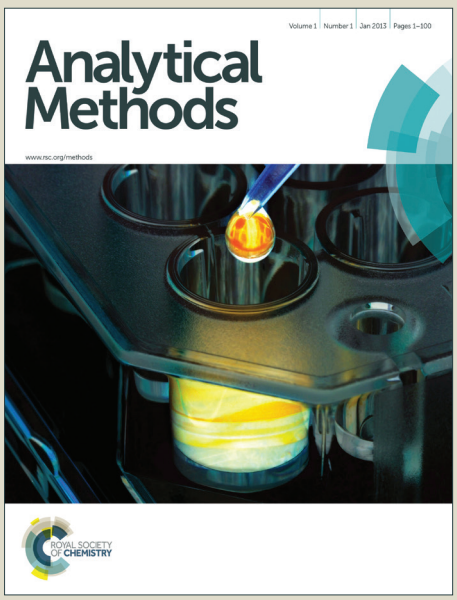

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Rapid and nondestructive evaluation of fish Freshness by near infrared reflectance spectroscopy combined with chemometrics analysis

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Abstract

Rapid and nondestructive measurement of freshness is essential for control of fish and its products' quality and safety. In this study, K value was measured by high performance liquid chromatography (HPLC) and employed as an index of fish freshness. The prediction models of the silver chub freshness were developed using Fourier Transform Near Infrared Reflectance Spectroscopy (FT-NIRS) with Several Partial Least Squares (PLS, i-PLS, Si-PLS), Support Vector Machines Regression (SVMR) and Synergy interval plus Support vector machine regression leading to Si-SVMR. By comparison, the performance of Si-SVMR model was superior to the others for the prediction of K value, where RMSECV = 0.027095 and Rc = 95.59% for calibration set, while RMSEP = 0.036525 and Rp = 93.74% for prediction set. The results indicated that FT-NIR spectroscopy together with Si-SVMR model could be a reliable method for detection of fish freshness.

Keywords: NIRS, fish freshness, K value, SVMR

1.0 Introduction

It is well known that freshness is the primary characteristic of the quality of freshwater fish and a positive property well evaluated by consumers. Loss of freshness and spoilage of fish are complicated processes and various factors such as fishing, handling, bleeding, storage temperature, the kind of fish species, the amount of food in the guts influence the spoilage pattern. The traditional detection methods of fish freshness mainly include sensory evaluation, microbiological, chemical, and

physical testing methods. Sensory evaluation methods based on significant appearance parameters such as, skin, slime, eyes, gills, belly and odor are conducted by experienced and well-trained humans(Nollet and Toldra 2010). Although these methods can be done without damage to the fish, these are subjective due to the subjective opinion of human judges and difficult to make quantitative analysis(Pons-Sánchez-Cascado, Vidal-Carou et al. 2006). At present microbiological methods based on total viable counts, chemical and biochemical methods (total volatile basic nitrogen, trimethylamine, pH, ATP, etc.), and physical testing methods play a key role in industrial fish quality and safety evaluation and inspection. Even certain of them have been used as regulation methods and gold standards serving scientific researches with their reliability and accuracy (Watanabe, Tamada et al. 2005, Alimelli, Pennazza et al. 2007, Zaragozá, Ribes et al. 2012). However, these methods are normally expensive, time-consuming, laborious, destructive and difficult for the application of the real-time detection. Recently emerging sensor array technologies, such as electronic tongue，electronic nose and olfaction visualization technology, were also used for the study of freshness detection. These methods have their advantages. Nevertheless, gas gathering, complicated testing procedures and moisture sensibility weaken the overall performance for electronic nose technology. Electronic tongue technology is not suitable for online testing due to the troublesome liquid sample preparation. As for olfaction visualization technology, the colorimetric sensor array preparation is a time-consuming effort and expensive metalloporphyrins directly result in the increase of detection cost (Gil, Barat et al. 2008, Huang, Xin et al. 2011, Ruiz-Rico, Fuentes et al. 2013). Therefore, it is necessary to develop a simple, fast, non-destructive, objective, economical and quantitative method for the detection of fish freshness (Wei and Wu 2007, Dowlati, de la Guardia et al. 2012).

Near infrared reflectance spectroscopy (NIRS) is the high-tech analytical techniques in the field of analytical chemistry. It is the integration of multi-disciplinary knowledge, such as spectroscopy, chemometrics, and computer multi-disciplinary knowledge and others. This method is simple, fast, green, low cost and has a good reproducibility. The root of modern near infrared analytical techniques

began earlier in the 1960s by the American Scientist Norris for Agriculture product quality measurement. This technology was usually used for a measurement of agricultural moisture, fat, protein and other substances(Khodabux, L'Omelette et al. 2007, Tito, Rodemann et al. 2012). With the rapid development of computer technology and the stoichiometry, the technology has been applied to various walks of life, such as pharmaceutical analysis(Porfire, Rus et al. 2012)， chemical engineering(Ribeiro, Raja et al. 2014)，environmental science (Buerck, Roth et al. 2001)，agricultural products quality researches (Nicolaï, Beullens et al. 2007).

The previous research objects of the detection of fish freshness with NIRS mostly were with fish fillets and surimi (Bøknæs, Jensen et al. 2002, Xu, Huang et al. 2012), with the fish body broken. Recently the back of the entire fish was also studied (Zhang, Xu et al. 2011, Li, Wang et al. 2013). Remarkably, the back of fish is covered by fish scales which are mainly composed of CaO，P_2O_5 and Collagen(Atta 2013). Normally, Inorganic Chemical (CaO，P_2O_5) has reflection interference on NIRS. Besides, NIRS may be also interfered with the different distribution of organs and fishbone in the abdominal cavity of fish. NIRS detection technology of large yellow croaker by removing fish scales based on PLS methods was proposed (Zhang, Xu et al. 2011). The result is ideal, but the method is also destructive. In contrast, the structure of fish eye is sample and easy to locate(Dowlati, Mohtasebi et al. 2013), which bring about a good test repeatability. Fish eye gradually becomes cloudy from the clear, until the corruption and collapse the storage duration, which is a sound determination indicator of fish freshness for consumers and fish merchants. Hence, NIR detection method based on fish eye could be rapid, simple, nondestructive and easy to locate in theory.

Recently, a new perspective that K value is a more accurate index for the early freshness of fish than TVBN (Total Volatile Basic Nitrogen) was put forward. In the view, the freshness of fish depends primarily on its own biochemical reactions. That a series of biochemical reactions occurred after death is the essence of the change of fish freshness and has nothing to do with the microbial decomposition. The microbial decomposition occurred in the late stage of fish deterioration. The decomposition of

ATP in fish meat sets in after death, and its compounds are subsequently produced according to the following sequence.

ATP → ADP → AMP → IMP → HxR(inosine)→ Hx(hypoxanthine)

The K value represents the proportion (%) of the total amount of inosine and hypoxanthine relative to that of ATP-related compounds. The higher the K value, the less fresh the fish. ATP had a rapid degradation from the dead to rigor mortis, leading to a sharp increase of K value. Hence, K value is a more accurate index for the early freshness of fish than TVBN(Etsuo Watanabe 2005). The researches on horse mackerel(Losada, Piñeiro et al. 2005), trout (Bizri, Bouhours et al. 1985), yellow fin tuna(Kamalakanth, Ginson et al. 2011) and other during iced storage(Lougovois, Kyranas et al. 2003) showed that K value could be used as an important chemical indicator for the evaluation of fish freshness.

Freshwater fish is popular with consumers because of its delicacy and nutrition. Silver carp (Hypophthalmichthys molitrix) is a widespread species of freshwater fish in China. In this study, NIRS was attempted for the detection of K value in silver carp during storage and preservation. In addition, HPLC was employed for K value measurement of silver carps. Multiple spectral preprocessing methods were compared. PCA algorithm was applied for dimensionality reduction. Several partial least square techniques were applied (PLS, i-PLS, Si-PLS). Support Vector Machine Regression and Synergy interval plus Support vector machine regression leading to Si-SVMR were also acquired for the quantitative prediction of K value of silver carp.

2.0 Materials and methods

2.1Silver carp samples

180 fresh silver carps (*Hypophthalmichthys molitrix*) were purchased from Zhenjiang fish market with an average weight of 950 g and average body length of 40 cm. The fish were washed with running water and then put into food preservation kits with labels (1~180). Then all fish were stored in a refrigerator at about 4℃. 15 fish were selected randomly for the spectral collection every day and then they were slaughtered for the measurement of K values. The test was conducted for 12 days.

2.2. NIR instrumentation and data collection

The spectrum of each sample was collected in the reflectance mode using the Antaris II Near Infrared Spectrophotometer (Thermo Electron Company, USA) with an integrating sphere. Built-in background was as a reference. Each eye of the fish was scanned 5 times. The average of 10 data was used as the final raw data of the fish. The spectra of 15 fish were collected one by one every day. Each time a fish was taken out of the fridge for the spectral collection. After the spectral collection of the fish was finished, it was transferred to the next laboratory in an ice box for the measurement of K value. The whole experiment was conducted at an ambient temperature of 25 ± 1℃ and the air humidity was kept at steady state. Each spectrum was an average of 16 scans with a spectral range of 10,000–4000 cm^{-1}, and the raw data set were measured in the interval of 8 cm^{-1}, resulting in 1557 variables. The raw NIRS profile of a silver carp is shown in Fig.1.

2.3. Determination of K value

K value is an indicator of the fish freshness. The researches on horse mackerel(Losada, Piñeiro et al. 2005), trout(Bizri, Bouhours et al. 1985), yellow fin tuna(Kamalakanth, Ginson et al. 2011) and other during iced storage(Lougovois, Kyranas et al. 2003) showed that K value could be used as an important chemical indicator for the evaluation of fish freshness. It equals to the percentage of the sum of inosine and hypoxanthine and the total of adenosine triphosphoric (ATP) and its decomposition product. Expressed as(Losada, Piñeiro et al. 2005, Kimiya, Sivertsen et al. 2013):

$$K(\%)=\frac{100\times(HxR+Hx)}{ATP+ADP+AMP+IMP+HxR+Hx} \quad (1)$$

ATP—adenosine triphosphate ,

ADP—adenosine diphosphate ,

AMP—adenosine monophosphate,

IMP—inosine monophosphate,

HxR—inosine,

Hx—hypoxanthine .

All concentration unit were ’μmol / g’.

The fish was immediately slaughtered for the measurement of K values after its near-infrared spectral collected. Specific steps are described below:

The flesh of fish back without scales was ground into minced fish with the mincing machine. 1g of minced fish was homogenized with 10mL of purified water for 1 min. 20mL of 15% PCA(perchloric acid solution) was added to the mixture , keeping on stirring for 5 min. Then the sample was centrifuged and supernatant was poured into a beaker (100mL). The sediment was washed by 5% PCA and further was centrifuged. Afterwards, the first and the second supernatant were merged. Repeat the above twice.

The supernatant was neutralized (pH 6.8) by adding 5M KOH , standing in an ice bath for 30 min. Then the solution was centrifuged and the supernatant was poured into 100-mL volumetric flask. The sediment was washed by 5% PCA-KOH. The first and the second supernatant were merged and transferred into the above volumetric flask. Repeat the above twice.

Finally, the solution in the 100-mL volumetric flask was diluted with purified water to 100 mL and agitated, standing in the an ice bath in a short time.

Centrifugation conditions: 9000r/min, 10℃, 10min.

Liquid chromatography conditions:

1) high performance liquid chromatography (Ultimate 3000 DIONEX company in Germany), C18 reverse phase column (Shimadzu inertsustain C18 4.6 × 150 mm, 5μm),

2) mobile phase: 0.05M disodium hydrogen phosphate-the phosphodiesterase sodium hydroxide buffer (pH 6.8),

3) detection time : 30 min,

4) detection wavelength: 254 nm,

5) flow rate: 0.8mL/min,

6) column temperature: 2600℃.

The types and concentration of the single standard and mixed standard solution of ATP , ADP, AMP, IMP, HxR and Hx were measured under the same conditions. The standard curve was drawed. Fig.2. shows High Performance Liquid Chromatogram (HPLC) of the standard ATP-related compounds. The types and concentration of the standard samples were determined by comparing the retention time and peak area of samples with standards. K value was calculated according to the method described elsewhere(Kimiya, Sivertsen et al. 2013). The variation trend of K value of silver chub during the cold storage was shown in Fig. 3.

2.4 Chemometric methods and evaluation index

All computations, chemometric analysis and graphics were executed with programs developed in Matlab 2008b (The Mathworks, Inc., Natick, MA, USA). The SVMR models were fitted using the functions provided in the libsvm-mat-2.89-3 toolbox.

The chemometric techniques was used in the research, including Savizky－Golay polynomial curve smoothing (SG), 1st derivation(D1), Principal Component Analysis

(PCA), Support Vector Machine Regression (SVMR) and multifarious Partial Least Squares (PLS, i-PLS, Si-PLS).

The application of SG-D1 is beneficial not only for the removal of noise but also for extending the differences of characteristics(Xu, Xie et al. 2011, Chen, Ding et al. 2012). Compared with Partial Least Squares (PLS), Synergy Interval Partial Least Squares (Si-PLS) needs the less number of spectra variables but the result is not less than the result from PLS model in general(Chen, Zhao et al. 2007, Godoy, Vega et al. 2014, Teye, Huang et al. 2014). Support Vector Machine (SVM) determines the appropriate trade-off between learning ability of limited samples and the learn accuracy of specific samples for the best generalization performance (Devos, Ruckebusch et al. 2009, Alves and Poppi 2013).

Root mean square error of cross-validation (RMSECV), Root mean squared error of prediction (RMSEP) and Correlation coefficient (R) were employed as index for the performance of the achieved model. The smaller RMSECV and RMSEP were, the stronger the predictive ability of the achieved model was. The higher the similarity of measurements and prediction values will make the R output move further towards 1, leading to the stronger predictive ability of the achieved model(Chen, Zhao et al. 2009, Teye, Huang et al. 2013).

2.5 Calibration and prediction set

On the first day, due to the difference of the silver carp vitality, the time of their death was different after they were placed into the refrigerator. That brought large differences on K value of the first day. Some fish stayed alive corresponding to K value but others did not. Considering that the freshness of dead fish was studied in the paper, the data from the next day to the twelfth day (a total of 11 days) were used for modeling. The samples were divided into two sets, namely calibration set and prediction set. The calibration set was made up of 110 samples and these samples were used to model. 55 samples were used as the prediction set, which was used to test the reliability and stability of the model. To minimize the error and avoid bias in the division of the subsets, the data is rearranged according to the size of K value from low to high order. Then in every 3 samples, two were selected as calibration set

and 1 as the prediction set.

2.6 Quantification models

Multifarious Partial Least Squares (PLS, i-PLS, Si-PLS), SVMR and Si-SVMR models were applied for the prediction of K value of silver carps during the cold storage. Firstly, multiple pretreatment methods and combination between them were compared, including Savitzky-Golay (SG), First Derivative (D1), Second Derivative (D2), Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC) and Mean Centre (MC) as well as the combination of SG and D1. The selection principle was based on maximal correlation coefficient (R). Then PLS with the full spectrum, i-PLS and Si-PLS quantitative models were respectively achieved. And the efficient joint intervals were also generated by Si-PLS model. Next, Principal Component Analysis (PCA) was used for dimensionality reduction and then SVMR model with the full spectra and the optimal combined subintervals from Si-PLS model were respectively obtained.

3.0 Results and discussion

3.1K value analysis

ATP in the fish flesh would be degraded as: ATP→ADP→AMP→IMP→HxR→Hx after the death of fish. K value is more accurate to indicate the changes in the early stage (freshness) of fish than TVBN (Total Volatile Basic Nitrogen). Because the rapid ATP decomposition leads to the soar of K value. However, the rate of protein degradation is slow from the death of fish to final decay. The lower the K value, the more fresh the fish. Inversely, the higher the K value, the less fresh the fish. There are many studies on the relationship between K value and fish freshness (Lougovois, Kyranas et al. 2003, Losada, Piñeiro et al. 2005, Kamalakanth, Ginson et al. 2011). It is generally acknowledged that K value of live fish is not more than 10%; K value of the secondary freshness range from 20% to 40%; the fish is inedible/harmful when the K value is more than 40% (Khodabux, L'Omelette et al. 2007).

In this work, the behavior of K value during cold storage is shown in Fig.1. K value is on the rise with the storage duration, with a sharp increase especially in the first three days. The silver carps were fresh food with an average K value of 10.64%

on the first day. K values of some fish were less than 10%, indicating that the fish were still dying on the first day. And K value of the death on the first day ranged from 10% to 20%, suggesting that they were fresh. On the second day silver carps were also fresh with average K value of 19.67%. The silver carps were the secondary freshness with an average K value of 20%～40% from the third to seventh days. The fish is inedible starting from the eighth day. The perception of spoilage happened on the twelfth day(Kimiya, Sivertsen et al. 2013).

3.2 NIR examination

Many factors have an effect on spectral response values in the process of measurement, including high frequency random noise, baseline drift, signal to background, uneven concentration, light scattering, or the optical path change and others. Spectra preprocessing is very important in near infrared spectroscopy analysis for the decrease of error. Multiple preprocessing methods were investigated combining with Si-PLS model, including Savizky-Golay polynomial curve smoothing (SG), 1st derivation (D1), 2nd derivation (D2), Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC) and Mean centering (MC) as well as SG-D1. A SG filter with five smoothing points was employed to smooth and remove random noise from NIR spectra. D1 and D2 of the spectra were also used to increase the spectral resolution and to solve problems of baseline shift and linear tilt. MSC and SNV were used to correct both multiplicative and additive effects of the spectra due to scattering, light scattering, particle size, and the change of optical path. In MSC, each spectrum was linearized to the average spectrum. Moreover, SNV was conducted by normalizing each individual spectrum to zero mean and unit variance (Jamshidi, Minaei et al. 2012). The arbitrary origin of interval scale variables can be eliminated by MC. From Tab.1, the result of SG-D1 was better than others with maximum correlation coefficient (R). So SG-D1 method was applied to the spectrum preprocessing in the study. The profile of the raw NIRS and corresponding SG-D1 of a fish is shown in Fig.2. It could be found that the raw spectra were smoothed and weak peaks were enhanced after the application of SG-D1 preprocessing methods.

3.3. Different PLS models

The full spectrum range from 4000 to 10000 cm^{-1} was used. PLS model with the full spectrum and iPLS model were obtained in sequence. The results are respectively shown in the Fig.4 and Fig.5, where R=0.8087 and 0.8017 respectively in the prediction sets. The performances of the two models were unsatisfactory, so Si-PLS model was further used. Si-PLS was divided into 15, 16, 17,…, 21 intervals combined with 5 to 9 subintervals as seen in Table 2. The optimal combination of intervals and the number of PLS factor was optimized by cross validation. The best performance was chosen according to the lowest root mean square error (RMSE). The best results obtained by Si-PLS are shown in bold and italic characters from Table 2. Scatter plots of Si-PLS model are shown in Fig.6. Seven spectra subintervals from the spectral regions were selected by Si-PLS model as seen from Fig.7, including 5142.9-5428.6 cm^{-1} , 6571.3-7142.7 cm^{-1} , 7428.4-7999.8 cm^{-1} and 8856.9-9428.3 cm^{-1}. All the ranges have 523 variables out of the 1557 variables. The selection was in accordance with the group active spectral region in general. The spectra between (5142.9-5428.6 cm^{-1}) showed peak at 5169 cm^{-1} was associated with O–H stretching and combination, which indicates the absorption peak of the water, at 5421 cm^{-1} associated with C–H first overtone. The spectra (6571.3-7142.7 cm^{-1}) were obtained at 6861 cm^{-1} and 6852 cm^{-1} both associated with C-H stretching, at 7030 cm^{-1} associated with O–H first overtone(Hunter, Kourtellis et al. 2011, Teye, Huang et al. 2014) , at 8000～8800 cm^{-1} associated with C–H second overtone(Atta 2013). These spectral regions are typical for fat, fatty acid and water in the fish(Atta 2013, Teye, Huang et al. 2014), which have a close relationship with the freshness.

3.4. SVMR models

SVMR model with synergy intervals partial least squares (to form Si-SVMR model) and SVMR model with the full spectra were also applied in this work. The top priority was the choice of kernel function for modeling SVMR. Because mapping the original data X nonlinearly into a higher dimensional feature space is implemented by a kernel function(Chauchard, Cogdill et al. 2004). RBF kernel was used in the study referring to the previous work (Gunasekaran, Paulsen et al. 1985). In order to get a better performance, the penalty parameter C and kernel parameter γ have to be

optimized. The optimal performance of SVMR models was superior with penalty parameter C =9.2674 and kernel parameter γ =0.01(setting by γ in kernel function RBF) in the work. PCA was used for data dimension reduction. Then 3 PCs of both the full spectra and the joint intervals were as the input of the models. The result of SVMR model with the full spectra is shown in Fig.8. SVMR model had a correlation coefficient of 0.9410 in the calibration set and 0.9192 in prediction set, with RMSECV=0.03177 and RMSEP= 0.03656 respectively, which displayed a good performance but under fitted in the prediction model. Besides, the result of Si-SVMR model was shown in Fig.9. From Fig.9, R =0.9595 for the calibration set and 0.9374 for the prediction set, with RMSECV =0.02710 and RMSEP = 0.03653 respectively, which indicated an excellent performance of the prediction model. The predictive values of Si-SVMR model and the measured K values from the prediction set are shown in Table 3. The average relative error is 0.084. The model has a relatively large error for the prediction of K value. But for the freshness discrimination the error is still relatively reasonable. The first fresh sample was predicted as the secondary freshness with K=0.2335. The 30th secondary fresh sample was estimated as the inedible object with K=0.4232. And 5 inedible samples were predicted as the secondary freshness (20% <K<40%). Then Si-SVMR model has an accuracy of 87% for the freshness discrimination. The prediction accuracy of K value near the critical value has a great influence for the freshness discrimination. The prediction accuracy of model still needs to be optimized.

As shown in Table 4. Si-SVMR was compared with different PLS models and SVMR model with the full spectra. It was obvious that i-PLS model had the worst generalization performance among the six. It is possible that some important information were left out with the single interval. In the contrast, PLS model with the full spectra which included all the spectra had more noise and redundant information, which deprived the performance of the model. Si-PLS model merged together the advantages of i-PLS and PLS, where as much as useful information remained and noises were eliminated. So Si-PLS model was better than i-PLS and PLS models. Even so, the performance of PLS models are inferior to SVMR models. One of

reasons is that two SVMR models belong to the non-linear recognition model while Si-PLS model used here belong to the linear recognition model (Godoy, Vega et al. 2014). Generally, nonlinear models have significantly stronger self-learning ability in the training process than the linear model. In fact there are also some potential nonlinear relationship between spectral data and chemical measurements. So the performance of the two SVMR models was better than the three PLS models. Si-SVMR model had the best generalization performance of the six. It is possible that the full spectra region contains more noise and redundant information, which weaken the predictive performance of the model. Conversely Si-SVMR selected multiple spectral sub-intervals that are informative and irrelevant information eliminated that could deprive the performance of the model. Besides, the Si-SVMR model with the less wave numbers is more efficient than SVMR model. When all the above factors are taken into account，NIRS combined with Si-SVMR model was more suitable for the detection of K value of fish.

4.0 Conclusions

This study shows that FT-NIRS coupled with chemometrics techniques could be used for the quantitative prediction of K value of silver chub during the cold storage. The eye of the silver chub is a feasible location for the detection by NIR spectroscopy, due to the simple structure of fish eyes, the constant location and close relationship with the fish freshness during the cold storage. The chemometrics treatment by (Si-PLS, SVMR and Si-SVMR) of spectral data was faster, simpler and more convenient than HPLC. Si-SVMR model had the best performance with a correlation coefficient of 95.59% in the calibration set and 93.74% in prediction set, which showed that NIRS together with Si-SVMR model has a high potential for the prediction of K value in silver chubs during the cold storage. Although the performance of Si-SVMR model needs to be further improved, the study could provide a reference for the quantitative analysis of the fish freshness by near infrared spectroscopy.

Acknowledgements

The authors wish to acknowledge the financial assistance provided by the

National Natural Science Foundation of China (No.31071549), Special Fund for Agro-scientific Research in the Public Interest (No.201003008), Priority Academic Program Development of Jiangsu Higher Education Institutes (PAPD).

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Tables

Table 1 The performance of Si-PLS model with different preprocessing methods

Methods	R_{cal}	R_{pre}	PCs
SG	0.8669	0.8675	8
SNV	0.8338	0.8117	8
D1	0.8753	0.8779	8
D2	0.8274	0.7731	8
MSC	0.8322	0.8156	8
Centralization	0.8669	0.8675	8
SG-D1	***0.8880***	***0.8776***	***8***

Table 2 Calibration results by Si-PLS model with different spectral range selection

Number of intervals	**PCs**	**Selected intervals**	**RMSECV**	**RMSEP**
15	8	1,4,9,10,12,13,5,7	0.0446	0.0504
16	8	4,8,9,10,13,14,1,7,11	0.0453	0.0512
17	8	4,8,9,11,14	0.0458	0.0489
18	8	4,5,8,9,7,15	0.0454	0.0481
19	8	5,10,9,13,12,17,16	0.0454	0.0499

20	8	5,9,13,11,16,17	0.0474	0.0464
21	***8***	***5,10,11 ,14,15,16,17***	***0.0421***	***0.0443***
22	8	5,6,1,12,10,13,14,18	0.0463	0.0515
23	8	6,11,12,14,19,23	0.0456	0.0503
24	8	6,11,13,16,20	0.0467	0.0496
25	8	6,12,13,16,21,1,5	0.0432	0.0553

Table 3 The error analysis of measured and predictive K values in Si-SVMR prediction set

Sample number	Measured K value	Predictive value	Absolute error	Relative error
1	**0.1910**	**0.2335**	**0.0425**	**0.2223**
2	0.2026	0.2089	0.0063	0.0312
3	0.2081	0.2150	0.0069	0.0331
4	0.2117	0.2699	0.0582	0.2750
5	0.2250	0.2010	0.0240	0.1067
6	0.2300	0.2151	0.0149	0.0648
7	0.2364	0.2843	0.0479	0.2028
8	0.2409	0.2609	0.0200	0.0830
9	0.2568	0.2686	0.0118	0.0461
10	0.2641	0.2723	0.0082	0.0312
11	0.2690	0.3255	0.0565	0.2101
12	0.2739	0.3316	0.0578	0.2109
13	0.2799	0.3335	0.0536	0.1915
14	0.2861	0.2968	0.0107	0.0375
15	0.2906	0.3053	0.0147	0.0505
16	0.2946	0.2975	0.0029	0.0099
17	0.2993	0.3470	0.0477	0.1593
18	0.3025	0.3148	0.0123	0.0408
19	0.3135	0.3547	0.0412	0.1313

20	0.3204	0.3272	0.0068	0.0212
21	0.3306	0.3341	0.0035	0.0107
22	0.3349	0.3526	0.0177	0.0528
23	0.3395	0.3504	0.0109	0.0321
24	0.3434	0.3736	0.0302	0.0878
25	0.3491	0.3714	0.0223	0.0640
26	0.3526	0.3887	0.0360	0.1022
27	0.3689	0.3958	0.0269	0.0730
28	0.3712	0.2989	0.0723	0.1948
29	0.3750	0.3701	0.0049	0.0131
30	**0.3791**	**0.4232**	**0.0441**	**0.1164**
31	0.3914	0.3610	0.0304	0.0777
32	0.3971	0.3798	0.0173	0.0436
33	**0.4055**	**0.3663**	**0.0392**	**0.0966**
34	**0.4075**	**0.3861**	**0.0214**	**0.0524**
35	0.4170	0.4133	0.0037	0.0088
36	**0.4214**	**0.3909**	**0.0305**	**0.0724**
37	**0.4292**	**0.3925**	**0.0367**	**0.0855**
38	**0.4333**	**0.3950**	**0.0382**	**0.0882**
39	0.4385	0.4342	0.0042	0.0097
40	0.4405	0.4116	0.0289	0.0656
41	0.4503	0.4095	0.0408	0.0905
42	0.4539	0.4727	0.0188	0.0414
43	0.4566	0.4077	0.0488	0.1070
44	0.4586	0.4266	0.0320	0.0698
45	0.4603	0.4242	0.0361	0.0784
46	0.4634	0.4071	0.0563	0.1215
47	0.4774	0.4409	0.0364	0.0763
48	0.4809	0.4573	0.0237	0.0492

49	0.4850	0.4797	0.0053	0.0109
50	0.4892	0.4103	0.0789	0.1612
51	0.4923	0.4757	0.0166	0.0337
52	0.4972	0.4176	0.0796	0.1600
53	0.5100	0.5203	0.0103	0.0202
54	0.5189	0.4813	0.0376	0.0724
55	0.5201	0.5104	0.0097	0.0187
Average value	0.3661	0.3635	0.0290	0.0840

Table 4 Results of training and predicting based on SVMR and Bi-PLS model

Regression model	number of variable	Principal component factors	The training set		The prediction set	
			r	RMSECV	r	RMSEP
PLS	1557	8	0.8289	0.0512	0.8087	0.0553
iPLS	92	5	0.8096	0.0532	0.8017	0.0554
Si-PLS	523	8	0.8880	0.0421	0.8776	0.0443
SVMR	1557	3	0.94104	0.031770	0.91919	0.036561
Si-SVMR	523	3	0.95947	0.027095	0.93743	0.036525

Figures

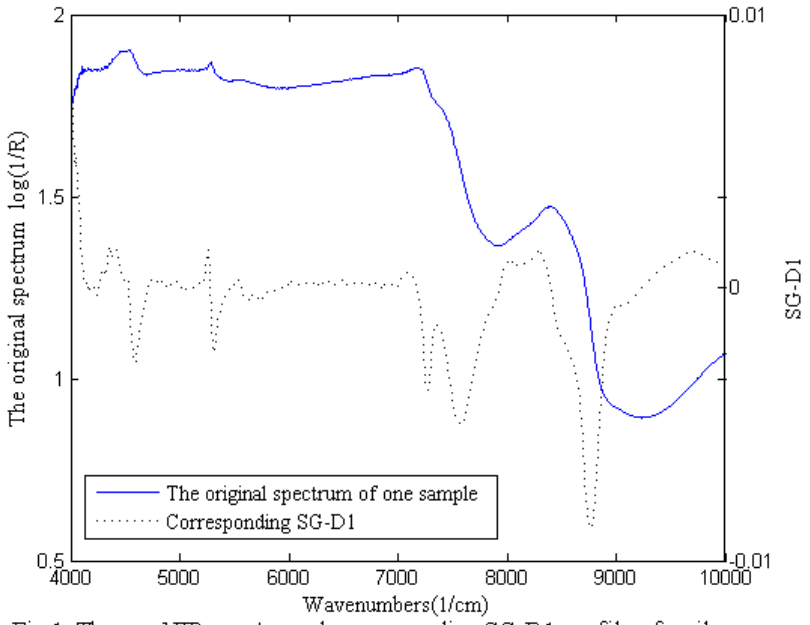


Fig.1. The raw NIR spectra and corresponding SG-D1 profile of a silver carp

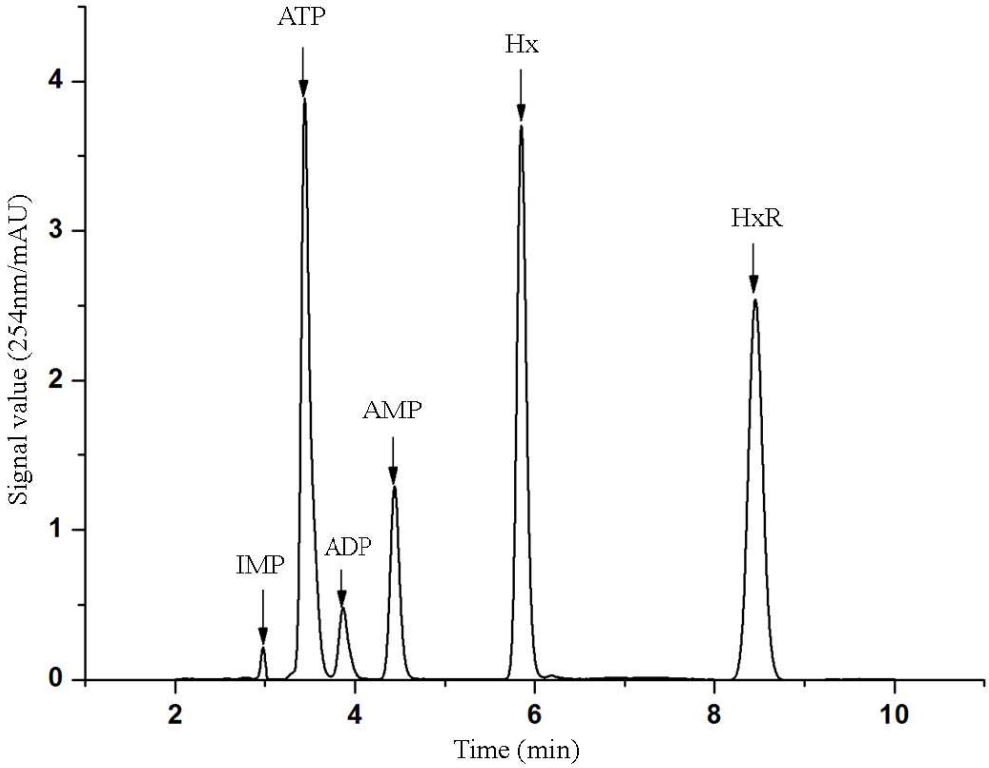


Fig.2. High Performance Liquid Chromatogram (HPLC) of the standard ATP-related compounds

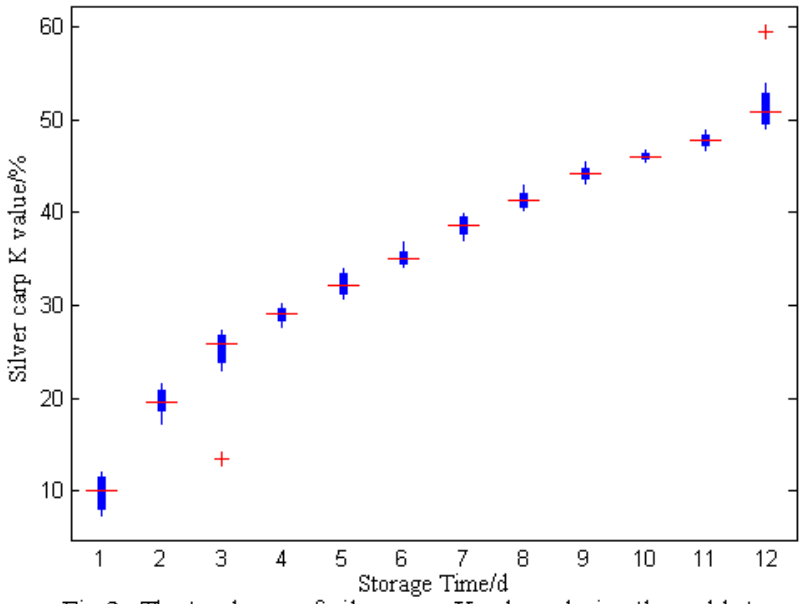


Fig.3. The tendency of silver carp K values during the cold storage

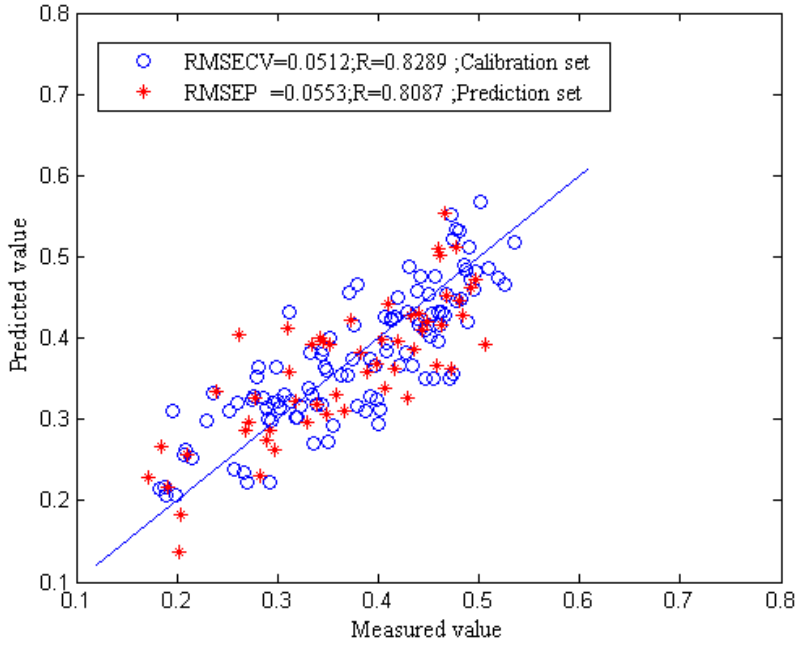


Fig.4. Scatter plots of PLS model with NIR estimation and experimental K value

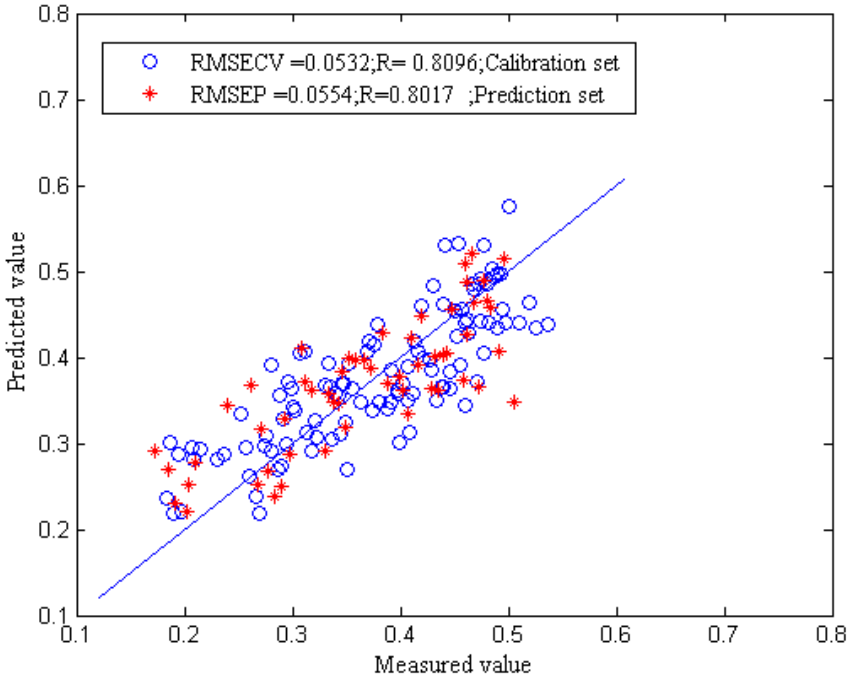


Fig.5. Scatter plots of i-PLS model with NIR estimation and experimental K value

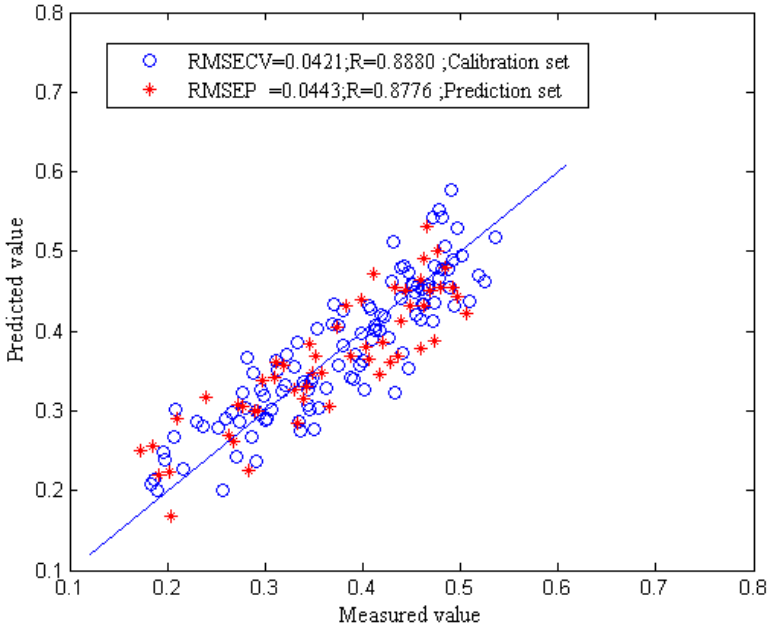


Fig.6. Scatter plots of Si-PLS model with NIR estimation and experimental K value

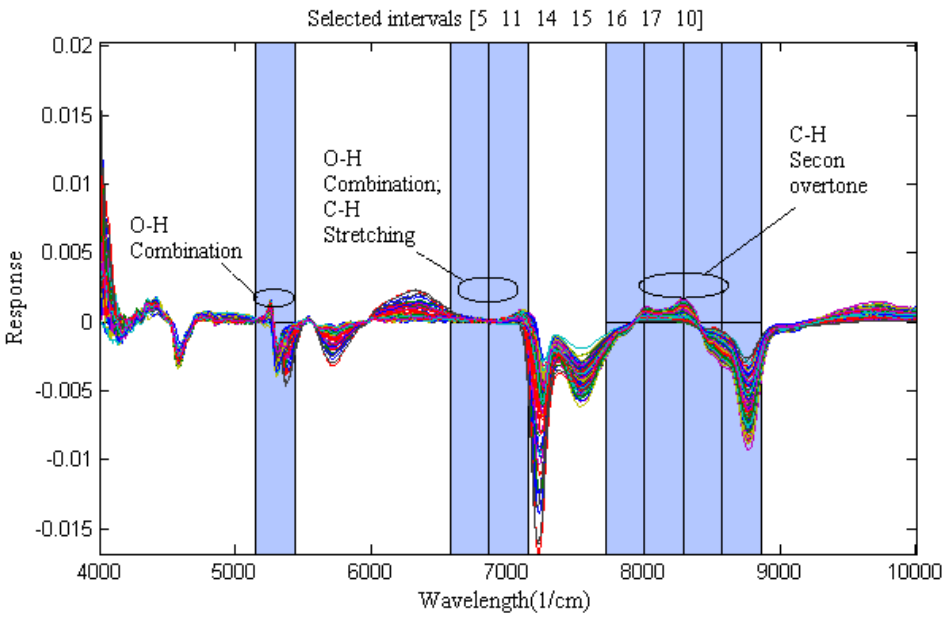


Fig.7. Si-PLS spectral selection at 8 PLS components

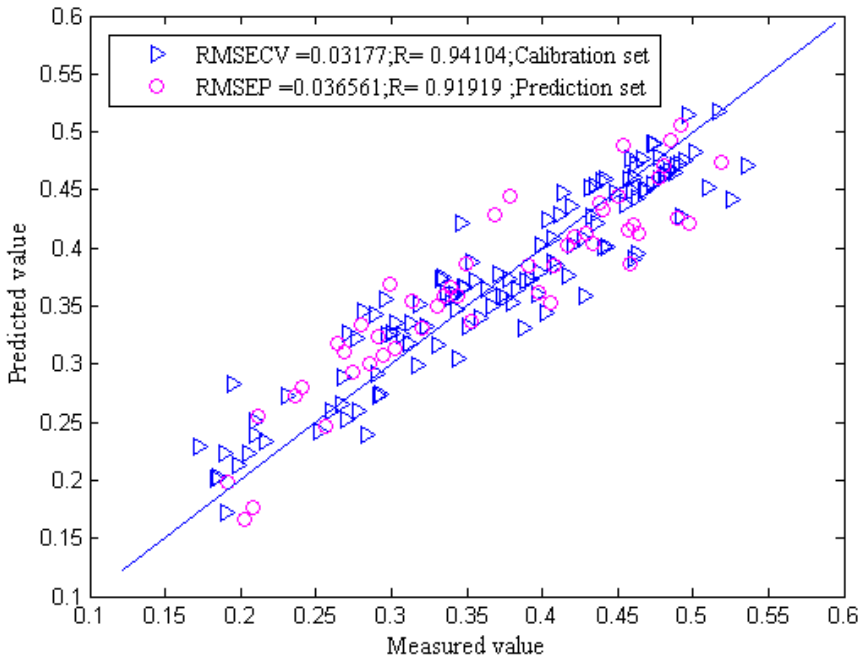


Fig 8. Scatter plots of SVMR model with NIR estimation and experimental K value

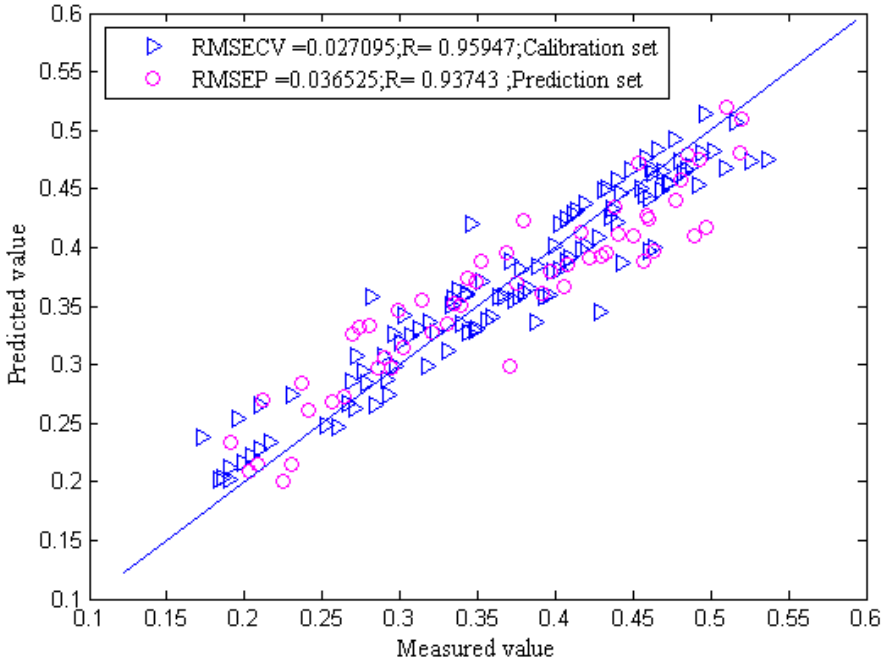
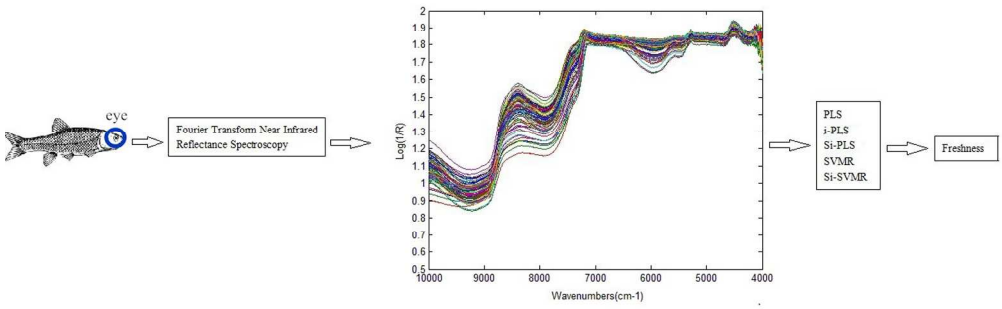


Fig 9. Scatter plots of Si-SVMR model with NIR estimation and experimental K value



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