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Prediction of amino acids in freeze dried pork by near infrared reflectance spectroscopy

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ABSTRACT

NIRS was used to predict the amino acid profile of freeze-dried pork samples. Samples (150; *Longissimus thoracis et lumborum*) of pork were used for analysis. After freeze drying, samples were analyzed using HPLC to find out the amino acid content. Samples were scanned and partial least squares (PLS) regression methods were used to predict the amino acid. The determination coefficient obtained by full cross-validated (80 as a sample for calibration set, 25 samples as a validation set) PLS models indicated that the NIR original spectra had an excellent ability to predict the contents of alanine, proline and methionine. Prediction of glutamic acid and glycine using standard normalized variate (SNV) pretreatment of spectral modeling was accurate. Similarly, prediction of arginine, tyrosine, valine, isoleucine, leucine, phenylalanine and lysine were accurate using SNV or multiplicative scattering correction (MSC) pre-processing spectra modeling. It was not possible to predict aspartic acid, serine, threonine, cystine, and histidine. These results indicated that the NIRS can be used for prediction of selected amino acids in the freeze dried pork.

Key words: Amino acids, Near infrared spectroscopy, Partial least squares, Pork

In recent years, the consumption of both meat and meat products has increased, which is primarily attributed to the improvement in living standards of people. People begin the pursuit of delicious and pay more attention to meat quality and safety (Dixit *et al.* 2017, Pieszczek *et al.* 2018). Amino acid content is one of the important indicators of quality and also as the meat important flavour precursors in pork (Dashdorj *et al.* 2015). Amino acids are known to be related to the development of particular desired or undesired taste, flavour or aroma. They also react with other metabolites and form diverse aromatic substances. Amino acid detection is usually by amino acid analyzer or HPLC which in turn results not only in higher estimation costs, takes more time but also results in destructive testing. NIRS being a kind of environment-friendly analysis technology of rapid, objective, non-destructive, and accurate was useful for simultaneous analysis of components in organic substances. It has been used for rapid detection of moisture content, protein content, fat content and fatty acids in livestock and poultry meat (Tao *et al.* 2013). There were more reports about protein forecast, but the few reports about amino acids.

NIRS has been successfully applied to the quantitative determination of the main chemical components such as moisture content, protein content, fat content and fatty acids in meat and meat products with high accuracy (Ripoll *et al.* 2018). Furthermore, NIRS enables fast and accurate prediction of the essential amino acid contents in wheat, maize, soy, cottonseed and fishmeal (Lin *et al.* 2009, Li *et al.* 2012, Du *et al.* 2016). However, only few reports are available for prediction of amino acids in meat. Some people tried to establish the NIRS model for prediction of free amino acids in dry-cured ham (Prevolnik *et al.* 2011). Results showed that threonine, serine, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and histidine obtained satisfactory predictions, but the tyrosine, arginine, glutamic acid, aspartic acid, proline, and cystine had poor prediction results. Reports regarding the prediction of amino acids using NIR spectroscopy are scarce in freeze dried pork samples.

We choose 105 samples of pork (*Longissimus thoracis et lumborum*) spectra modeling to analysis. This experiment adopted the wave number range to 4,000–10,000/cm of the near infrared spectrum. The coefficient of determination obtained by full cross-validated (80 samples as a sample for calibration set, 25 samples as a validation set) PLS to set up the amino acids of quantitative analysis models. These models of quantitative analysis have different spectral preprocessing methods, which find the optimal quantitative model of amino acids.

MATERIALS AND METHODS

Sample preparation: Fresh meat (*Longissimus thoracis*

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et lumborum of pork) samples (105) were obtained from Panlong garlic village of farmers' market in Kunming, Yunnan province. All samples used were mincing machine broken. Took these minced samples for freeze drying to constant weight, sieved through 80 mesh sieve and loading it in 5 wire 4 polyethylene valve bag to scanning spectra.

We used the HPLC method to detect the contents of amino acids. The HPLC method was described in compositional analysis, and the scanning spectrum was described in spectral acquisition.

Compositional analysis: Using the freeze-dried powder to detect the contents of amino acids in HPLC, samples were pre-treated by acid hydrolysis method. The instrument was Shimazu HPLC (LC-20A). Column chromatography detection conditions included amino acid analysis column specifications of 4.6×250 mm, 5 µm; column temperature of 40°C; sample quantity of 20 µl; wavelength of 254 nm; and the mobile phase flow rate of 1 ml/min.

Spectral acquisition: It was the acquisition of valve bag background spectrum and combined with the 2 mm solid fibre optic probe. Meat samples were scanned at 8/cm resolution for 30 times over the NIR spectral rang 4,000–10,000/cm using Shimadzu Fourier IRPrestige-21 transform infrared spectrometer with the near infrared in attachment (FlexIRTM NIR Fiber Optic Accessory). Samples were scanned 3 part and each part scanned 3 times in duplicate (resulting in 9 spectra/sample). Thus, the area of the sample scanned could be increased and the sampling spectra error could be reduced. The average spectrum was used for each sample. Absorbance data were stored as log(1/R),where R is the reflectance.

Spectral data processing: Calibrations were performed by PLS regression. To optimise the accuracy of calibration, several scattering corrections and mathematical treatments were tested (first derivative, second derivative, SNV, MSC) (Yan 2005).

First derivative formulas: $\frac{dy}{dx} = \frac{y_{i+1} - y_i}{\Delta x}$ or $\frac{dy}{dx} = \frac{y_{i+1} - y_i}{2\Delta x}$

Second derivative formulas: $\frac{d^2y}{dx^2} = \frac{y_{i+1} - 2y_i + y_{i-1}}{\Delta x^2}$

The average spectra: $\bar{A}_i = \frac{\sum_{i=1}^{n} A_i}{n}$

Linear regression: $\bar{A}_i = m\bar{A}_i + b_i$

MSC correction: $A_{i(MSC)} = \frac{(A_i - b_i)}{m_i}$

where, A, spectrum matrix of the correction; A_i for the I sample spectrum of I; m_i and b_i were the spectrum of A_i; and the average spectrum of A were linear regression of the slope and intercept. The data try to retained the original information related to the chemical composition that adjusted the m_i and b_i to reduced spectral differences at the same time.

SNV correction: $Z_i = \frac{x_i - \mu}{\sigma}$

Partial least squares (PLS) in near infrared spectrum analysis, are the most widely used method. PLS was the perfect combination of multivariate linear regression, canonical correlation analysis, and principal component analysis (Yan 2005, Lu 2010).

Samples were selected at random from 2 groups. Three-quarters of the total samples were used for calibration and full cross-validation (80 samples) and the other quarter (25 samples) was used to validate calibrations. We used the spectra of raw, first derivative, second derivative, SNV and MSC pre-treatment, and adopted PLS with full cross-validation to build the forecast model. We took the determination coefficient (R^2), root-mean-squares error of cross-validation (RMSECV), root-mean-square error of prediction (RMSEP) as a model precision evaluation index to determine the spectral preprocessing methods and the PLS-components (PCs). Thus, we use the residual predictive deviation (RPD) value to determine the model stability and reliability of prediction.

RESULTS AND DISCUSSION

Chemical composition of the tested muscles: The concentration ranges and standard deviations for each amino acids, in which it is possible to apply the calibration equations obtained with HPLC as the reference method are given in Tables 1, 2. We used the descriptive statistics for the amino acid content in freeze dried pork of *Longissimus thoracis et lumborum*. Eighty samples as calibration set and 25 samples as validation set. Table 1 has the amino acids calibration content. The measured values revealed that there

Table 1. The amino acid calibration content (freeze dried, %)

Amino acid	Minimum	Maximum	Mean	SD
Asp	7.42	9.92	8.59	0.52
Glu	11.39	15.03	13.02	0.83
Ser	2.63	3.52	3.08	0.19
Gly	3.30	4.36	3.78	0.24
His	2.69	7.01	5.44	0.84
Arg	4.79	6.33	5.57	0.41
Thr	2.92	3.82	3.38	0.21
Ala	4.22	5.21	4.70	0.24
Pro	2.40	3.13	2.77	0.16
Tyr	2.35	2.97	2.69	0.16
Val	3.82	4.61	4.19	0.20
Met	1.97	2.47	2.22	0.12
Cys	0.01	0.25	0.06	0.05
Ile	3.48	4.27	3.89	0.21
Leu	5.42	6.75	6.13	0.35
Phe	2.80	3.49	3.15	0.18
Lys	6.08	7.60	6.89	0.42

Aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cystine (Cys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys).

Table 2. The amino acid validation content (freeze dried, %)

Amino acid	Minimum	Maximum	Mean	SD
Asp	7.43	8.97	8.50	0.41
Glu	11.39	13.99	12.94	0.78
Ser	2.61	3.49	3.02	0.22
Gly	3.3	4.07	3.76	0.24
His	4.35	6.97	5.35	0.85
Arg	4.79	6.13	5.54	0.43
Thr	2.93	3.76	3.34	0.24
Ala	4.22	5.08	4.68	0.25
Pro	2.31	3.06	2.73	0.21
Tyr	2.35	2.96	2.66	0.20
Val	3.82	4.51	4.16	0.23
Met	1.78	2.42	2.17	0.17
Cys	0.01	0.13	0.05	0.03
Ile	3.48	4.26	3.86	0.25
Leu	5.42	6.69	6.09	0.39
Phe	2.8	3.43	3.11	0.21
Lys	6.08	7.4	6.77	0.43

Aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cystine (Cys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys).

were wide variations in the 17 measured components. Table 2 has the amino acids validation content.

NIR spectral interpretation in freeze dried pork samples: The calibration of raw spectra about freeze-dried pork samples is shown in Fig. 1. Spectra of freeze dried pork samples after MSC pretreatment is shown in Fig. 4. Fig. 5 shows the spectra of freeze dried pork samples after SNV pretreatment. These show the noise of 4,000–4,300/cm and 9,000–10,000/cm were larger than other spectrum periods that hard to see the absorption peak clearly. Figs 1,4,5 had a high absorption peak on 4230, 4300, 4580, 4860, 5800, 6620 and 8340/cm. The spectra information defines a series of characteristic absorption bands.Thus, the C–H bond, which is a fundamental constituent of amino acids molecules, absorbs clearly at wavelengths close to 4230, 4300, 5800, 7150, 8340/cm (Willians and Norris 1987). Moreover, the 4230–4300 region corresponds to the combination bands of the C–H bond, and the absorption produced in the 5680–5800 region corresponds to the first overtone of that bond (Workman and Weyer 2007). In the current research, 7080–7200 region had an absorption peak. It was the absorption peak of C–H bond.

Table 3. Effect of different pretreatments on calibration models

Amino acids	Pre-treatment	R_c^2	RMSEC	RMSECV (%)	RMSEP (%)	PCs (%)	RPD
Asp	None	0.09	0.49	0.51	0.33	5	1.25
	1-Derivative	0.23	0.45	0.5	0.35	2	1.20
	2-Derivative	0.23	0.45	0.5	0.34	2	1.20
	SNV	0.31	0.43	0.51	0.36	3	1.15
	MSC	0.31	0.43	0.51	0.36	3	1.15
Ser	None	0.43	0.14	0.16	0.15	3	1.43
	1-Derivative	0.42	0.14	0.16	0.16	2	1.39
	2-Derivative	0.42	0.14	0.16	0.16	2	1.40
	SNV	0.42	0.14	0.16	0.16	2	1.36
	MSC	0.42	0.14	0.16	0.16	2	1.36
His	None	0.48	0.65	0.69	0.50	2	1.68
	1-Derivative	0.46	0.72	0.81	0.50	2	1.70
	2-Derivative	0.51	0.63	0.73	0.51	2	1.68
	SNV	0.50	0.64	0.75	0.40	3	2.13
	MSC	0.50	0.64	0.75	0.40	3	2.12
Thr	None	0.48	0.15	0.17	0.18	3	1.37
	1-Derivative	0.44	0.16	0.17	0.19	2	1.26
	2-Derivative	0.44	0.16	0.18	0.19	2	1.26
	SNV	0.55	0.14	0.18	0.18	3	1.30
	MSC	0.54	0.14	0.18	0.18	3	1.31
Cys	None	0.01	0.05	0.05	0.03	1	1.00
	1-Derivative	0.05	0.04	0.05	0.03	1	0.98
	2-Derivative	0.05	0.04	0.05	0.03	1	0.96
	SNV	0.11	0.04	0.05	0.04	1	0.87
	MSC	0.11	0.04	0.05	0.04	1	0.87

Fig. 2 shows the spectra of freeze-dried pork samples after first derivative pretreatment (Gap size was 1, Segment size was 1). Fig. 3 shows the spectra of freeze dried pork

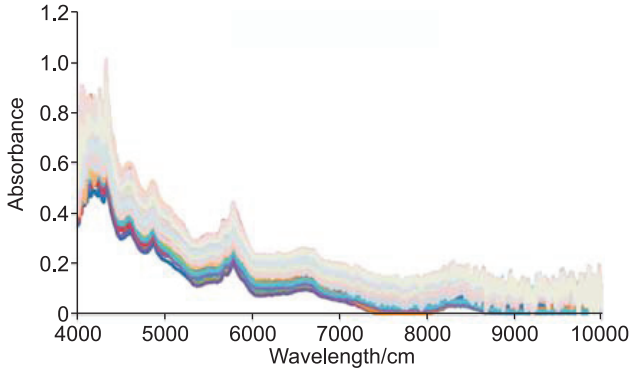

Fig. 1. Raw spectra about freeze-dried pork samples.

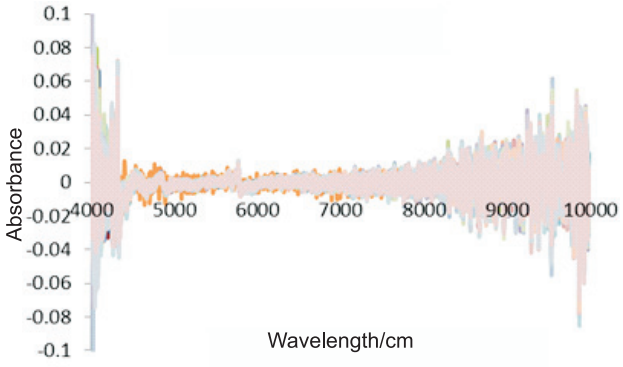

Fig. 2. First derivative spectra of the freeze-dried pork samples.

Table 4. Effect of different pretreatments on calibration models

Amino acids	Pre-treatment	Rc^2	RMSEC (%)	RMSECV (%)	RMSEP (%)	PCs	RPD
Ala	None	0.73	0.12	0.19	0.12	6	2.12
	1-Derivative	0.4	0.18	0.21	0.20	2	1.25
	2-Derivative	0.4	0.18	0.21	0.20	2	1.26
	SNV	0.82	0.1	0.19	0.13	7	1.95
	MSC	0.81	0.1	0.19	0.13	7	1.95
Pro	None	0.80	0.07	0.12	0.10	8	2.06
	1-Derivative	0.11	0.15	0.15	0.20	1	1.04
	2-Derivative	0.11	0.15	0.15	0.20	1	1.03
	SNV	0.78	0.07	0.12	0.09	6	2.26
	MSC	0.77	0.07	0.12	0.09	6	2.25
Met	None	0.85	0.05	0.09	0.08	8	2.00
	1-Derivative	0.38	0.1	0.11	0.14	2	1.17
	2-Derivative	0.38	0.1	0.11	0.14	2	1.17
	SNV	0.84	0.05	0.09	0.08	7	2.10
	MSC	0.84	0.05	0.09	0.08	7	2.10

samples through second derivative pretreatment (Gap size was 1, Segment size was 1). Figs 2, 3 were similar in attenuate the spectra absorption peak. The derivative can remove baseline drift and flat the influence of background interference. However, in the current research, the absorption peak of the spectrum was weakened.

The characteristics of the protein band were nearly 9804–10277/cm that the N-H stretching vibration of the secondary frequency. But in the current experiment, this period of the spectrum have the high noise that we could not see obvious absorption peak. The band of near 6667–6536/cm also characteristics that the protein N-H stretching vibration of first level frequency multiplication. We could observe obvious absorption peak on the band of near 6620/cm in raw spectra, MSC spectra, and SNV spectra. The bands of 4878–4854/cm have the absorption about N-H combination frequency of the stretching vibration. In the current research, the raw spectra, MSC spectra, and SNV spectra have an obvious absorption peak on 4860/cm.The obvious absorption peak on 4592/cm was combination tone about the curve of frequency multiplication (N–H), telescopic (C=O), in-plane bending (N–H), stretching vibration (C–N) (Workman and Weyer 2008).

Table 5. Effect of different pretreatments on calibration models

Amino acids	Pre-treatment	Rc^2	RMSEC (%)	RMSECV (%)	RMSEP (%)	PCs	RPD
Glu	None	0.37	0.66	0.72	0.56	3	1.38
	1-Derivative	0.34	0.67	0.74	0.59	2	1.31
	2-Derivative	0.34	0.67	0.74	0.58	2	1.33
	SNV	0.71	0.47	0.72	0.36	7	2.17
	MSC Derivative	0.43	0.62	0.74	0.58	3	1.33
Gly	None	0.69	0.13	0.2	0.09	7	2.59
	1-	0.34	0.19	0.21	0.17	2	1.37
	2-Derivative	0.34	0.19	0.21	0.17	2	1.40
	SNV	0.71	0.13	0.21	0.10	3	2.31
	MSC	0.43	0.18	0.22	0.15	7	1.59
Arg	None	0.69	0.23	0.25	0.17	3	2.47
	1-Derivative	0.71	0.22	0.24	0.20	2	2.16
	2-Derivative	0.7	0.22	0.24	0.19	2	2.24
	SNV	0.74	0.21	0.25	0.21	3	2.04
	MSC	0.74	0.21	0.25	0.21	3	2.04
Tyr	None	0.85	0.06	0.11	0.09	8	2.12
	1-Derivative	0.42	0.12	0.14	0.15	2	1.31
	2-Derivative	0.42	0.12	0.14	0.15	2	1.31
	SNV	0.85	0.06	0.11	0.09	7	2.14
	MSC	0.85	0.06	0.11	0.09	7	2.12
Val	None	0.82	0.08	0.15	0.12	8	1.95
	1-Derivative	0.12	0.19	0.2	0.23	1	1.01
	2-Derivative	0.12	0.19	0.2	0.23	1	1.00
	SNV	0.79	0.09	0.16	0.11	6	2.08
	MSC	0.79	0.09	0.16	0.11	6	2.06
Ile	None	0.74	0.1	0.16	0.14	6	1.83
	1-Derivative	0.15	0.19	0.2	0.23	1	1.09
	2-Derivative	0.14	0.19	0.2	0.24	1	1.06
	SNV	0.84	0.08	0.16	0.12	7	2.01
	MSC	0.84	0.08	0.16	0.13	7	2.00
leu	None	0.69	0.19	0.24	0.18	5	2.16
	1-Derivative	0.45	0.26	0.29	0.30	2	1.33
	2-Derivative	0.45	0.26	0.29	0.29	2	1.34
	SNV	0.87	0.12	0.24	0.17	7	2.36
	MSC	0.86	0.13	0.24	0.17	7	2.34
Phe	None	0.66	0.11	0.14	0.19	5	1.11
	1-Derivative	0.4	0.14	0.16	0.17	2	1.20
	2-Derivative	0.4	0.14	0.16	0.17	2	1.21
	SNV	0.85	0.07	0.13	0.10	7	2.01
	MSC	0.85	0.07	0.13	0.10	7	2.01
Lys	None	0.66	0.24	0.31	0.20	5	2.14
	1-Derivative	0.4	0.32	0.36	0.34	2	1.25
	2-Derivative	0.4	0.32	0.37	0.34	2	1.25
	SNV	0.86	0.16	0.3	0.20	7	2.19
	MSC	0.85	0.16	0.3	0.20	7	2.17

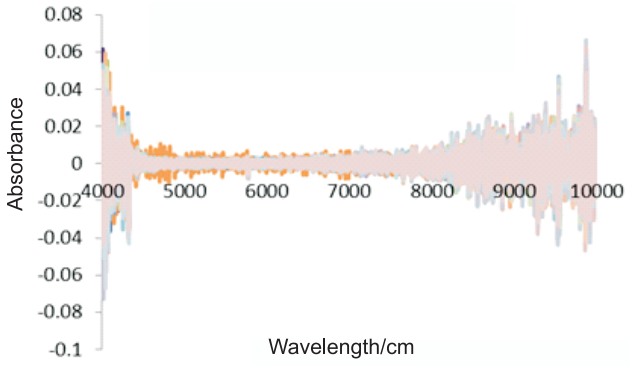

Fig. 3. Second derivative spectra of the freeze-dried pork samples.

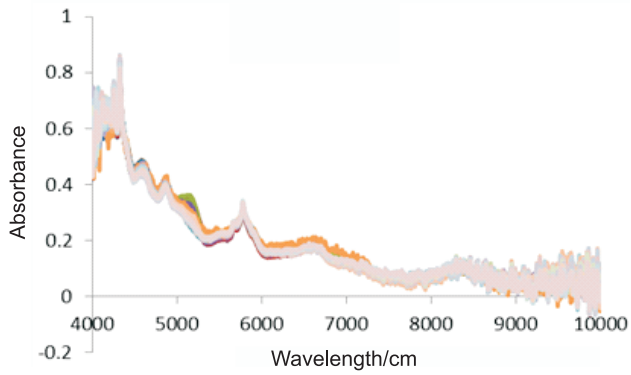

Fig. 4. MSC spectra of the freeze-dried pork samples.

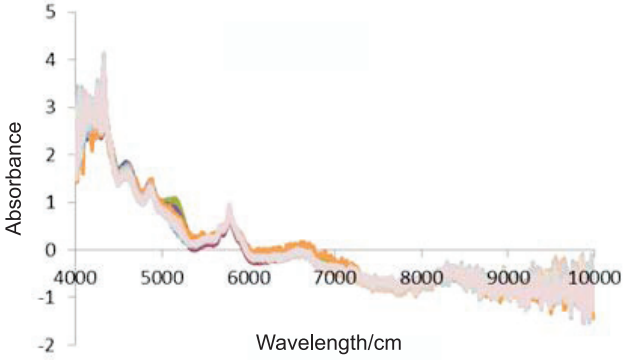

Fig. 5. SNV spectra of the freeze-dried pork samples.

PLS regression models for predicting amino acids: The Rc^2 of aspartic acid, serine, cystine, threonine and histidine were lesser than 0.55 (Table 3). The RPD of histidine was higher than 2 when used the SNV and MSC pretreatment, but the R_c^2 only 0.50. Thus, these 5 kinds of amino acids modeling were poorer that cannot be used for the actual forecast.

To reduce the dimensionality and compress the original spectral data, the principal components (PCs) obtained from principle component analysis (PCA) were considered as new eigen vectors of the original spectra.The number of PCs selected in PLS will affect the performance of PLS model, which needs cross validation in model calibration for optimization. The optimal quantity is based on the maximum value of R_c^2 and RPD, the lowest value of RMSECV. Generally, the PCs of the model is 6 to 9, which can control the risk of spectral noise and avoid excessive model fitting and inaccurate prediction of unknown samples (Brereton 2007). However, in this study, the number of selection was less than 5. We also tried to select the PCs greater than 5, and the effect was still not improved. Therefore, on the premise of the same effect of the model, we chose the minimum number of factors. May be it associated with the spectral choice spectrum. In the later study we will divide the spectrum to choice the model.

The calibrations with pretreatments about alanine, proline, and methionine are given in Table 4. The results showed that the raw spectra of these three amino acids had a good prediction model. The Rc^2 of these three amino acids values were 0.73, 0.80, 0.85 and the RPD were greater than 2. Thus, it has the strong robustness of the model. Alanine used the raw spectra prediction was good, and the Rc^2 was 0.73 that smaller than the 0.82 of SNV preprocessing spectra modeling. But the RPD of raw spectra was greater than 2. Thus, the model shows the strong robustness of the model. Therefore, the original spectra modeling of alanine was better. Barlocco *et al.* (2006) believed that RPD >2 was good for the pork calibration model. Other authors also think that RPD is more than two is the appropriate limit for quantitative forecasting, and can be applied to meat and most agricultural materials (Prieto *et al.* 2009).

The calibrations with pretreatments of glutamic acid, glycine, arginine, tyrosine, valine, isoleucine, leucine, phenylalanine and lysine are given in Table 5. After comparison, the SNV and the MSC pattern were considered as the proper spectral pretreatment. The results showed that glutamic acid and glycine used SNV spectra pretreatment were good. Arginine, tyrosine, valine, isoleucine, leucine, phenylalanine and lysine spectra modeling were good that used SNV or MSC preprocessing. In the near infrared diffuse reflection spectrum correction, MSC method can remove samples of mirror reflection and noise caused by non-uniformity, and it was also eliminating diffuse spectroscopy of baseline and the spectroscopy of non-reproducibility. SNV was to each spectrum for correction (Lu 2010), and the SNV approach effectively removes the multiplicative interferences of scatter and particle size. SNV could correct the error caused by the scattering spectrum between samples (Yan 2005).

The first derivative and the second derivative do not have a good prediction result; may be these related to the Gap size and Segment size. Different size will lead to the different pretreatment results. The first derivative and second derivative Gap-Segment derivative was applied with gap and segment sizes (Hopkins 2001). In the latter, we will study the different Gap size and Segment size to seek a better spectra pretreatment method. The influence of the spectrum noise and the random noise was generally high-frequency signal. It must demand that the original spectrum has a high signal-to-noise ratio them derivative would further enlarge the noise signal (Yan 2005). Thus, in this study, the first derivative and

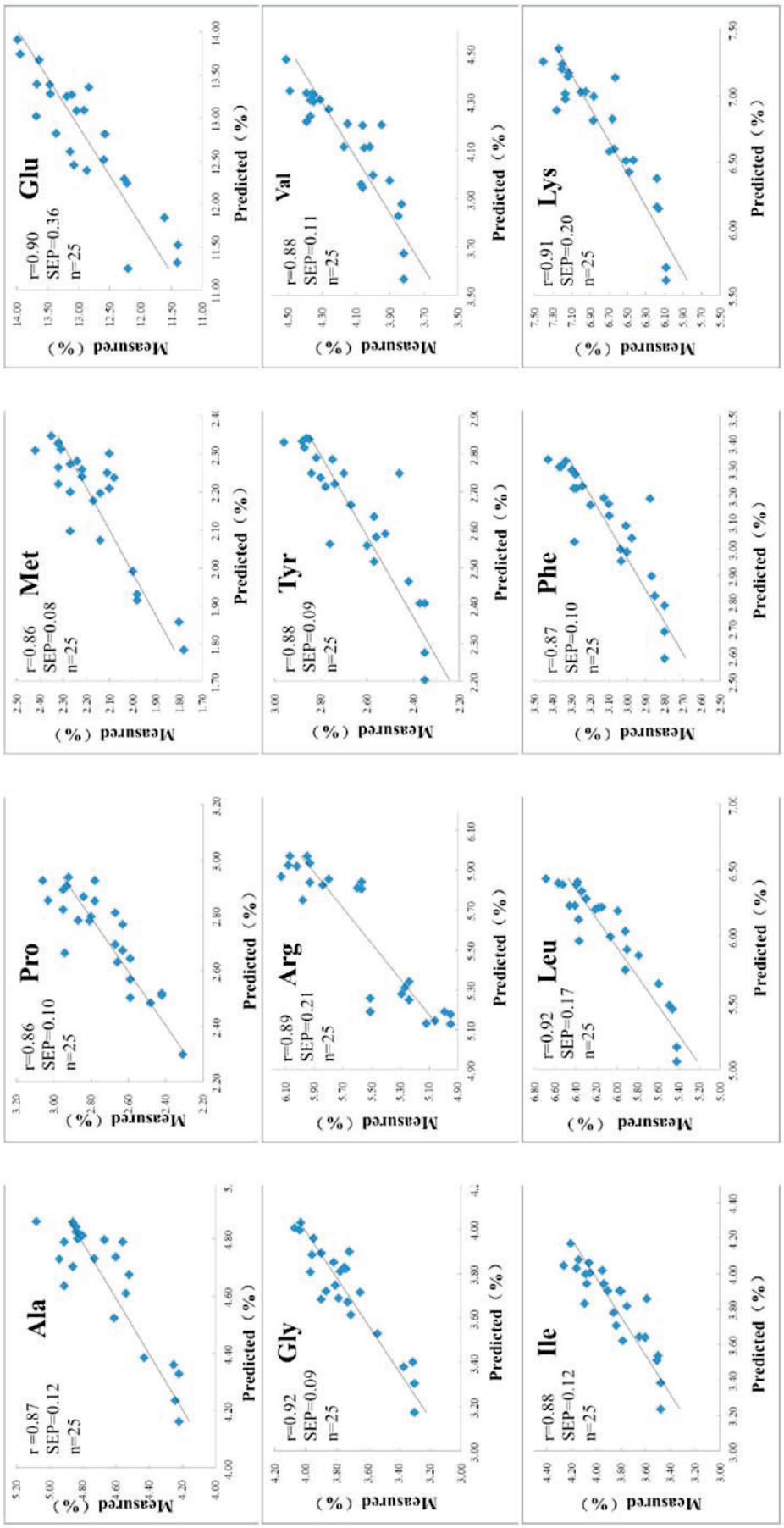

Fig. 6. The relationship between the predicted results of calibration model and the measured values of different amino acids.

second derivative pretreatment was not good. Maybe it is an indication of the faultiness in the methodology which was followed in the current research.

Prediction results of calibration model for forecast set: The correlation of the values obtained in the laboratory with respect to those predicted by NIR for the amino acids in *Longissimus thoracis et lumborum* of pork and the statistical descriptors of prediction are shown in Fig. 6. We used the calibration model of alanine, glutamic acid, valine, isoleucine, phenylalanine, glycine, arginine, proline, tyrosine, methionine, leucine, and lysine (Fig. 6) to forecast the 25 samples set of the forecast, and the correlation coefficient of r was greater than 0.86. From the r and SEP results, it may be inferred that the calibration model for these amino acids are robust and that it allows the determination of these amino acids with excellent results.

The NIRS was able to predict 15 different amino acids successfully through calibration and external validation statistics, except methionine and cysteine in brown rice (Zhang *et al.* 2011). In our study, methionine calibration model is better. It can carry on the forecast to the methionine, that the predicted and measured value correlation coefficient is 0.86. Prevolnik *et al.* (2011) reported that NIR spectroscopy could be applied for screening purposes of some free amino acids and total free amino acids in dry-cured ham Kraškipršut, for some amino acids (threonine, serine, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and histidine) satisfactory predictions were obtained. For these amino acids the R_c^2 were > 0.75 and RPD>2 which is above the value considered necessary for screening. But in our study threonine, serine, histidine and glycine prediction results were poor. In future studies, we will try different spectral processing methods to seek a better calibration model.

In conclusion, alanine, proline, and methionine used the raw spectra prediction model were better; glutamic acid and glycine used the SNV pretreatment prediction model were better; arginine, tyrosine, valine, isoleucine, leucine, phenylalanine and lysine used the SNV or MSC pretreatment prediction model were also better. The original or pretreatment of aspartic acid, serine, threonine, cystine, and histidine calibration model were not ideal. These results indicated that the NIRS can be used for prediction of selected amino acids in the freeze dried pork.

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