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Determination of Amino Acids in Chinese Rice Wine by Fourier Transform Near-Infrared Spectroscopy

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Chinese rice wine is abundant in amino acids. The possibility of quantitative detection of 16 free amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine) in Chinese rice wine by Fourier transform near-infrared (NIR) spectroscopy was investigated for the first time in this study. A total of 98 samples from vintage 2007 rice wines with different aging times were analyzed by NIR spectroscopy in transmission mode. Calibration models were developed using partial least-squares regression (PLSR) with high-performance liquid chromatography (HPLC) by postcolumn derivatization and diode array detection as a reference method. To validate the calibration models, full cross (leave-one-out) validation was employed. The results showed that the calibration statistics were good (r_{cal} > 0.94) for all amino acids except proline, histidine, and arginine. The correlation coefficient in cross validation (r_{cv}) was >0.81 for 12 amino acids. The residual predictive deviation (RPD) value obtained was >1.5 in all amino acids except proline and arginine, and it was >2.0 in 6 amino acids. The results obtained in this study indicated that NIR spectroscopy could be used as an easy, rapid, and novel tool to quantitatively predict free amino acids in Chinese rice wine without sophisticated methods.

KEYWORDS: Chinese rice wine; FT-NIR spectroscopy; amino acids; quantitative detection; partial least-squares regression; HPLC

INTRODUCTION

Chinese rice wine, also named yellow wine, has been one of the most popular alcoholic beverages in China for centuries. Fermented from glutinous rice and wheat using unique brewing techniques, Chinese rice wine is abundant in amino acids, proteins, oligosaccharides, vitamins, mineral elements, etc., and thus it is honored as the national banquet alcoholic beverage in China (*1*). Rice wine is not only for drinking but also for medical use in traditional Chinese medicine and has been claimed to have beneficial effects for the prevention of cancer and cardiovascular disease (*2*, *3*). Amino acids are important both as essential components of proteins and for their roles in energetic metabolism, neurotransmission, and lipid transport. Amino acids in Chinese rice wine mainly come from a hydrolysis reaction (catalyzed mainly by acid protease and acid carboxypeptidase) of proteins and microorganisms in glutinous and wheat koji, which serves as a source of nitrogen during alcoholic fermentation (*4*). Amino acids not only are nutrient components of Chinese rice wine but also are precursors for aroma compounds and directly contribute to the flavor of rice wine. Amino acids may taste fresh, sweet, bitter, or astringent, which brings Chinese rice wine a rich taste and enables the wine to be mellow, rich, soft, smooth, harmonious, multifragrant, and so on. For different foods and beverages, the amino acid profiles vary according to different varieties or origins (*5*). Therefore, the concentration of amino acids in Chinese rice wine can be used to evaluate the general quality of rice wine and may also be used to detect adulteration or fraud or to identify the origin or variety of rice wine. Hence, the precise qualitative and quantitative analysis of amino acids in Chinese rice wine is required.

The precise determination of amino acids in wines is complicated, because they are often present in small quantities. Therefore, sophisticated methods and instrumentations have been applied for decades. Several different techniques are used for the quantitative determination of amino acids in wines, and those most frequently used are ion-exchange chromatography with postcolumn derivatization using ninhydrin or *O*-phthalaldehyde (OPA) as derivatizing agent and ultraviolet (UV) detection, precolumn derivatization reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection, or separation of volatile amino acid derivatives by gas chromatography (GC) and detection by flame ionization detection (FID) (*6*). Although chromatographic techniques have a high accuracy, they are often time-consuming and destructive and require tedious and complex processing for samples, which limit their

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applications to rapid detection and process control. The use of chemical reagents is also a factor due to economic costs and safety considerations. Several works have been reported using HPLC for free amino acids analysis in Chinese rice wine. However, the results obtained in those works can only be used as reference and are not universal due to the small sample size. Hence, the development of rapid and cost-effective techniques for amino acid measurement is required when large numbers of samples have to be screened.

Near-infrared (NIR) spectroscopy together with chemometrics has gained wide acceptance in the fields of food industry and agriculture in recent years, mainly because it is a low-cost, nondestructive method and generally requires minimal sample processing prior to analysis (*7*). NIR spectroscopy can record the response of chemical bonds in functional groups (e.g., O−H, C−H, and N−H bands) to the NIR spectrum, which is related to the primary structural components of organic molecules. Quantitative NIR spectroscopy measurement is based on the correlation between sample composition, as determined by reference methods, and the absorption of NIR radiation by bonds between light atoms at different wavelengths in the NIR region (*8*). However, the absorption peaks of NIR spectra are broad and overlap, and it is impossible to make direct quantification analysis due to the high dimension and complexity of NIR spectral data. Chemometrics methods, such as principal component analysis (PCA), principal component regression (PCR), and partial least-squares regression (PLSR), are often used to extract spectral features and investigate the correlation between the spectra and component concentrations (*9*). At present, several studies have used NIR spectroscopy to determine different groups of compounds in wines. It has been used to determine five enological parameters (alcoholic degree, pH, total acid, amino acid nitrogen, and °Brix) and four oligosaccharides (isomaltose, isomaltotriose, maltose, and panose) in Chinese rice wine (*8*, *10*), screen 15 parameters (alcoholic degree, volumic mass, total acidity, pH, volatile acidity, glycerol, total polyphenol index, reducing sugars, lactic, malic, tartaric and gluconic acids, color, tonality, total sulfur dioxide, and free sulfur dioxide) in different types of wines (red, rose, and white wines) (*11*), measure volatile aroma compounds in Riesling wine (*12*), determine phenolic compounds in red wine fermentations (*13*), analyze different elements in red wines (*14*), determine fermentative volatile compounds, oak volatile compounds, and ethylphenols in aged red wines (*15*, *16*). In these papers, the compounds analyzed were found in wines at levels from micrograms per liter to grams per liter. The results suggested that NIR spectroscopy was suitable not only for the prediction of enological parameters in wines but also for the analysis of different chemical compounds in small amounts. However, no reports were found in relation to the use of NIR spectroscopy for the quantitative detection free amino acids in wines. Therefore, the aim of the present study was to investigate the possibility of Fourier transform near-infrared spectroscopy (FT-NIR) for the quantitative prediction of free amino acids in Chinese rice wine without sophisticated methods.

MATERIALS AND METHODS

Samples. A total of 98 Chinese rice wine samples were provided by three Shaoxing wine breweries (29 from Pagoda, 36 from Kuaijishan, and 33 from Guyuelongshan). Shaoxing rice wine is the most well-known rice wine in China, which is known by the generic name "Shaoxing", a sort of denomination of origin. The vintage year of all the Chinese rice wine samples was 2007. The samples had different aging times (22 samples, 3 months; 26 samples, 9 months; 24 samples, 11 months; and 26 samples, 15 months). The samples used in this study were all semidry type and selected from different batches of production for each sample and were directly taken from storage containers without any additive.

Table 1. Elution Gradient Used in the HPLC Method

time (min)	eluent A (%, v/v)	eluent B (%, v/v)	eluent C (%, v/v)
0	100	0	0
12	100	0	0
34	0	100	0
53	0	100	0
53.1	0	0	100
55	0	0	100
55.1	100	0	0
70	100	0	0

Reagents. All reagents used in this study were of analytical reagent grade. One milliliter of a standard mixture of 17 amino acids was purchased from the National Institute of Metrology (China), and the concentration for each amino acid was 1000 μg/mL. The 17 amino acids used as standards were aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg), and cystine (Cys). The postcolumn derivatization reagent ninhydrin and mobile phase reagents were all purchased from Pickering Laboratories, Mountain View, CA.

Instrumentation and HPLC Conditions. Amino acid analysis was performed by an 1100 series HPLC (Agilent Technologies Inc., Santa Clara, CA) combined with a postcolumn derivatization system model PCX 5200 (Pickering Laboratories). The HPLC system consisted of an online degasser, a quaternary gradient pump, an automatic sampler, and a diode array detector (DAD). The principle is similar to ion-exchange chromatography with postcolumn derivatization. Chromatographic separation was performed on a sodium ion exchange column (150 mm × 4.0 mm, i.d., 5 μm, Pickering Laboratories). Another sodium ion exchange column (20 mm × 3.0 mm, i.d., 5 μm, Pickering Laboratories) was used as guard column. Analyses were done using sodium eluent (1700-0112) as mobile phase A, sodium eluent (Na740) as mobile phase B, and sodium regeneration reagent (RG 011) as mobile phase C. The eluent gradient is shown in **Table 1**. The column temperature was kept at 50 °C. The column eluent merged with the derivatization reagent ninhydrin pumped at their respective flow rate, and the mixture was heated in the reaction coil at 130 °C. The flow rate of mobile phase was fixed at 0.4 mL/min and at 0.3 mL/min for ninhydrin. The DAD for the derivative was set at 570 nm. A 1 mL aliquot of Chinese rice wine was diluted 100 times using distilled water, passed through a 0.22 μm porosity filter, and injected automatically. The injection volume was 10 μL. Quantitative determination was carried out by preparing a synthetic solution containing a known amount of amino acids and analyzing it in the same instrument. Calibration plots were made by analyzing standard solutions of amino acids in different gradients, and the areas obtained in the analysis of the samples were interpolated in the corresponding calibration graphs to estimate the concentration of amino acids presented in samples. The amino acids were quantified by the external standard method from peak areas. All experiments were conducted at room temperature. The chromatogram acquisition was performed with Agilent chemical workstation. The coefficients of determination (R^2) of the method were > 0.999 for calibration curves of all standards. For recovery rates, they ranged between 95.4 and 102.5%, which indicated a good quantitative analysis.

Spectral Measurements. Before NIR spectra acquisition, all of the samples were stored in the laboratory at 20 °C for 24 h. Samples taken from freshly opened bottles were scanned in transmission mode using an FT-NIR spectrometer (Thermo Electron Corp., Madison, WI), which was equipped with an interferometer, a wide band light source (quartz tungsten halogen, 50 W), and an InGaAs detector. Samples were scanned in a demountable liquid cell (Pike Technologies, Madison, WI) of 0.5 mm optical path length with air as the reference. The sample cuvette was cleaned with distilled water and wiped dry after each measurement to avoid cross-contamination. Spectra were collected using OMNIC software (Thermo Electron Corp.) and saved in absorbance format. Spectra were recorded in the range of 800−2500 nm. The mirror velocity was 0.9494 cm s^{-1}, and the resolution was 2 cm^{-1}. The spectrum of each

sample was the average of 32 successive scans. Air background was taken each hour. There was no need to record a background spectrum before each sample measurement because of the high stability of the background (*17*).

Chemometrics and Data Analysis. In this study, spectra were exported from OMNIC software to TQ analyst software (version 6.2.1, Thermo Electron Corp.) for spectral pretreatment and chemometrics analysis. The Student residual test and leverage were employed first to detect outliers before modeling (*18*). Calibration models were developed using PLSR, which is the most commonly used multivariate method for the evaluation of NIR spectra. PLS is a method that has no restriction in the number of wavelengths that can be selected for the calibration to make the model suitable to extract the maximum information from the spectra. The properties of PLS and examples of its use have been dealt with extensively elsewhere (*19*). Here, PLS models with 1−15 factors were investigated, and the optimum number of factors used in PLSR was determined by the lowest value of the predicted residual error sum of squares (PRESS) to avoid overfitting. According to Williams (*20*), a calibration model should contain at least 100 samples, and so, due to our rather small sample set, leave-one-out cross-validation was employed to evaluate the established models. Cross-validation has the advantage that all of the data available can be used to determine the calibration model, because no sample has to be held back in a separate validation set (*21*). Several studies, including that of Moron and Cozzolino (*22*), have shown that both procedures provided similar results. The performance of the calibration is assessed by the correlation coefficient in calibration (r_{cal}), root-mean-square error of calibration (RMSEC), correlation coefficient in cross-validation (r_{cv}), and root-mean-square error of cross-validation (RMSECV) (*10*, *23*). In addition, to evaluate the prediction ability of the calibration model, the value of the residual predictive deviation (RPD) was employed in our work. The RPD value is defined as the ratio of standard deviation (SD) to standard error of cross-validation (RPD = SD/RMSECV). Usually the higher the RPD value, the greater the ability of calibration model to predict the chemical composition in samples (*13*). Referring to the criteria used by other authors (*24*), RPD and r greater than 3.0 and 0.94, respectively, are considered to be indicative of excellent prediction, whereas values from 2.5 to 3.0 (RPD) and from 0.89 to 0.94 (r) denote a good prediction. Approximate quantitative predictions are indicated by RPD values between 2.0 and 2.5 and r values in the range from 0.81 to 0.90. The possibility to distinguish between high and low values is revealed by values between 1.5 and 2.0 (RPD) and 0.70 and 0.81 (r). Unsuccessful predictions have RPD value and r value lower than 1.5 and 0.70, respectively.

RESULTS AND DISCUSSION

Chromatographic Analysis. Figure 1a shows the chromatogram of the standard mixture of 17 amino acids. All of the amino acid peaks were clearly separated with an analytical time of < 60 min. Ammonia (Amm), which was produced in derivatization, also showed a peak around 40 min. **Figure 1b** shows the chromatogram of one Chinese rice wine sample. All of the amino acids were detected in the sample except Cys, and the retention times in the chromatographic profile are consistent with the retention times in the standard mixture.

The statistics of amino acids in Chinese rice wine samples are summarized in **Table 2**. The sum of amino acids (sum (AA)) content in Chinese rice wine ranged from 2527.5 to 5290.4 mg/L in this study with an average concentration of 3749.2 mg/L; similar results were obtained by other authors (*4*, *25*). The results also indicated that Chinese rice wine contained more amino acids than other types of wines or vinegars (*26*−*28*). As can be seen in **Table 2**, Ala was found to be the most abundant amino acid, and its mean concentration was above 400 mg/L, consistent with the result of Wei (*29*). Glu, Pro, Leu, and Arg were found to be the second most abundant amino acids in Chinese rice wine, and the mean concentrations of the four amino acids were all > 300 mg/L. The first five amino acids represented 50.4% of the total amino acid content and mostly contributed both sweet and bitter tastes

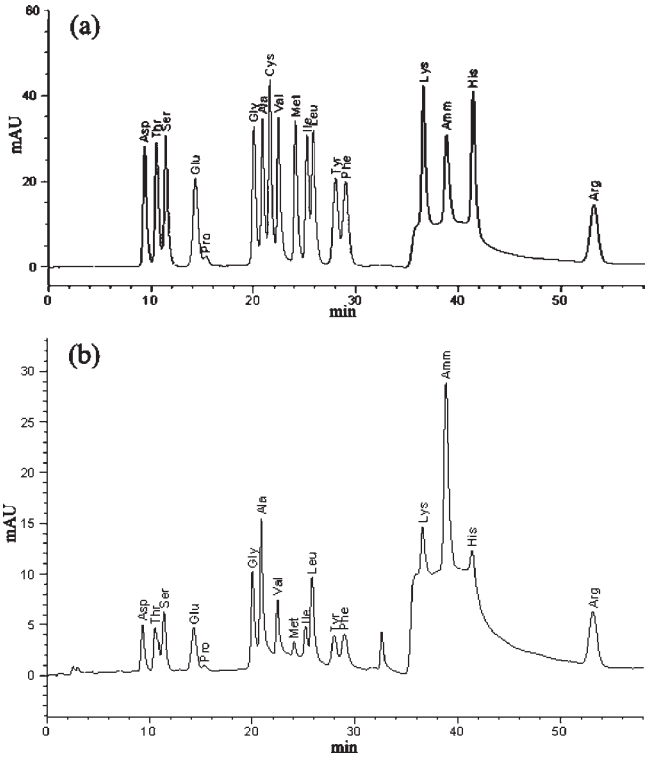

Figure 1. Chromatograms showing the amino acid profiles of (**a**) a standard mixture of 17 amino acids and (**b**) one Chinese rice wine sample.

Table 2. Descriptive Statistics of 16 Amino Acids in 98 Chinese Rice Wine Samples (Milligrams per Liter)

	max[a]	min[b]	mean	SD[c]
Asp	353.86	128.19	213.57	43.90
Thr	190.72	55.04	101.95	29.57
Ser	396.85	117.94	209.28	50.26
Glu	604.68	198.71	352.96	85.60
Pro	736.20	194.53	381.36	92.47
Gly	297.64	131.95	194.69	29.29
Ala	612.76	298.94	427.69	61.39
Val	231.79	122.68	165.56	21.86
Met	61.36	4.93	30.29	14.68
Ile	162.10	99.95	127.09	13.74
Leu	484.19	245.50	341.99	48.55
Tyr	305.35	88.22	216.29	49.10
Phe	362.02	197.82	273.79	33.69
Lys	405.66	93.90	209.14	58.07
His	242.17	45.37	118.16	45.25
Arg	720.54	142.59	385.35	129.92
sum (AA)[d]	5290.4	2527.5	3749.2	491.30

[a] Max = maximum. [b] Min = minimum. [c] SD = standard deviation. [d] Sum (AA) = sum of amino acids.

to Chinese rice wine. The concentration of Met was the lowest, with a mean value of only 30.29 mg/L. Cys was not detected using this assay and may be present in trace amounts according to the work of other authors (*25*, *29*). The remaining 10 amino acids had average values ranging from 100 to 300 mg/L.

Spectral Analysis. The original NIR absorbance spectra of 98 samples are shown in **Figure 2**. Wavelength regions with absorbance values of > 1.5 will not be used in any further analysis, which is beyond the linear response region of the detector (*30*). Generally, the main absorption bands were found at 1460 and 1934 nm, which were related to the first overtone of O−H group in water or carbohydrate and a combination of stretching and

deformation of O−H in water and ethanol, respectively (*31*, *32*). Absorption at 1692 nm might be related with $-CH_3$ stretch first overtone or C−H groups in aromatic compounds; absorption at 1776 nm was related with C−H stretch first overtone. Absorption at 2266 nm was likely related with C−H combinations and O−H stretch overtones. Absorption at 2302 nm was mainly related with C−H overtones of ethanol (*33*, *34*).

PCA. PCA is often the first step of data analysis to detect patterns or outliers in the data. In this study PCA was performed on the full spectral region to reveal any possible grouping of samples. **Figure 3** shows the score plot of the first two PCs derived from raw spectra, which account for 99.6% of the total variation (95.2 and 4.4% for PC1 and PC2, respectively). It was observed that the samples were separated clearly according to different aging times. It was possible that the aging of wines caused a variation in chemical components, which resulted in different spectral attributes. Several samples from aging time of 11 months were away from their group, which indicated a diversity of the aging course. However, no obvious groups between different breweries were observed in this study, which might due to similar climate or winemaking techniques.

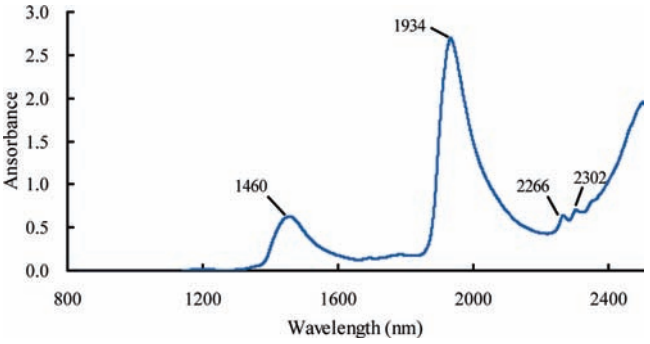

Figure 2. Original NIR absorbance spectra of Chinese rice wine samples.

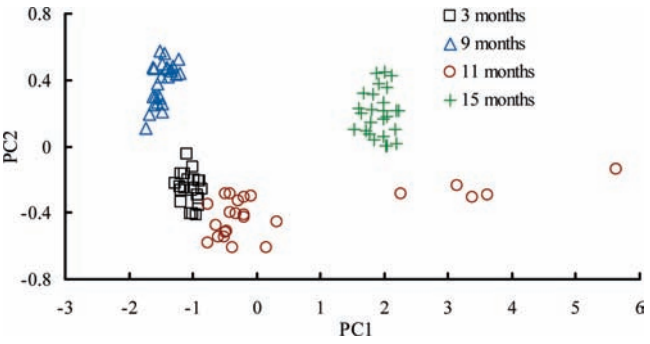

Figure 3. Two-dimensional principal component score plot derived from raw spectra.

PLSR. The PLSR models developed on the raw spectra were better than those on second-derivative spectra (data not presented), which is similar to results on Chinese rice wine published previously (*10*, *35*). Thus, raw spectra were employed for the calibration and validation analysis. The use of narrow spectral ranges for calibration models led to poor results in this study; thus, the full spectral range was applied to the quantitative analysis for 16 free amino acids in Chinese rice wine. Calibrations were developed using PLSR and leave-one-out validation. The choice of the model was based on the RPD value. The calibration statistics for the determination of amino acids using PLSR are summarized in **Table 3**. Student residual and leverage values were calculated to detect outliers before calibration. If there are samples with Student residual and leverage values that are noticeably different from the values of the other samples, which indicates they may differ in some respects, they will be considered as abnormal and examined closely to determine whether they provided any useful information or if they must be removed. A detailed discussion can be found elsewhere (*8*). For each amino acid, different samples were removed as outliers, which are also shown in **Table 3**. The value for the correlation coefficient in calibration (r_{cal}) was >0.94 for all of the amino acids except Pro, His, and Arg, which indicated that the correlation between the values determined by HPLC and the values estimated by NIR calibration was good. The correlation coefficient in cross-validation (r_{cv}) was >0.90, and RPD values were close to or >2.5 for Leu and Lys, which indicated excellent prediction for the two amino acids. Approximate quantitative predictions were obtained for Asp, Ser, Ala, and Phe. The RPD values for the four amino acids were >2.0, and r_{cv} values were all >0.85. The ability to distinguish between high and low values was obtained for Thr, Glu, Gly, Val, Met, Ile, Tyr, and His. The RPD values obtained were between 1.5 and 2.0, and r_{cv} ranged from 0.757 to 0.859. The predictions of Pro and Arg were unsuccessful because the

Table 3. Calibration Statistics for Amino Acids Determined Using PLSR by NIR Spectroscopy

								equation of linear regression		
	r_{cal}	RMSEC (mg/L)	r_{cv}	RMSECV (mg/L)	RPD	factors	outliers	slope	intercept	bias
Asp	0.940	14.3	0.872	20.4	2.05	8	1	0.772	48.59	0.302
Thr	0.993	3.84	0.855	15.0	1.93	14	3	0.710	29.78	−0.482
Ser	0.962	12.3	0.876	21.7	2.07	10	2	0.762	47.74	−1.32
Glu	0.960	23.9	0.850	43.5	1.90	10	2	0.753	85.39	−0.946
Pro	0.350	86.5	0.262	89.6	1.04	8	2	0.094	345.32	0.121
Gly	0.961	6.70	0.859	12.3	1.97	10	3	0.746	48.43	−0.317
Ala	0.970	14.8	0.870	30.2	2.03	11	0	0.787	91.62	0.467
Val	0.966	5.61	0.847	11.6	1.88	11	0	0.751	41.11	−0.179
Met	0.988	2.28	0.816	8.48	1.73	13	1	0.709	9.00	−0.148
Ile	0.983	2.49	0.830	7.68	1.79	13	2	0.748	32.30	0.147
Leu	0.982	8.64	0.932	16.8	2.77	11	1	0.844	52.81	−0.158
Tyr	0.956	14.4	0.799	29.7	1.66	11	2	0.676	68.75	−1.33
Phe	0.973	7.55	0.893	14.6	2.24	11	1	0.784	58.78	−0.188
Lys	0.979	11.1	0.916	22.1	2.49	10	4	0.868	26.99	−0.341
His	0.871	21.1	0.757	28.7	1.51	7	3	0.676	38.39	−0.537
Arg	0.754	80.8	0.615	98.2	1.26	6	3	0.455	206.48	0.211
sum (AA)	0.959	129.0	0.879	218.0	2.11	10	3	0.794	761.54	−5.29

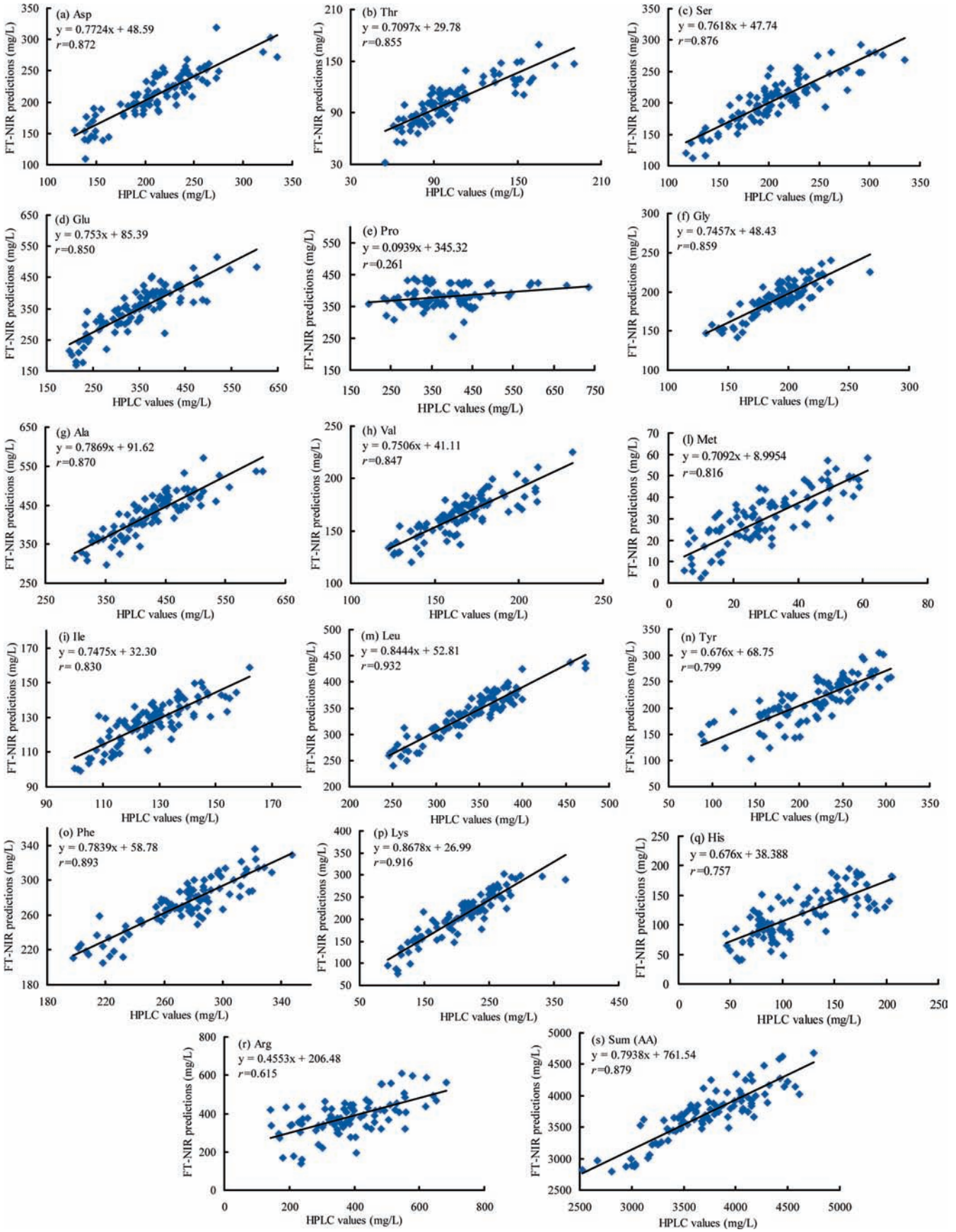

Figure 4. Correlation statistics between HPLC values and FT-NIR predictions by PLSR with leave-one-out validation.

RPD values were <1.5 and r_{cv} was <0.70 (0.262 and 0.615, respectively). In addition, according to the criteria using by other authors (*11*), RMSECV values of the models higher than RMSEC and lower than 2(RMSEC) are considered to have good precision. Therefore, the predictions of Asp, Ser, Glu, Gly, Leu, Phe, Lys, and His were all considered to be acceptable. However,

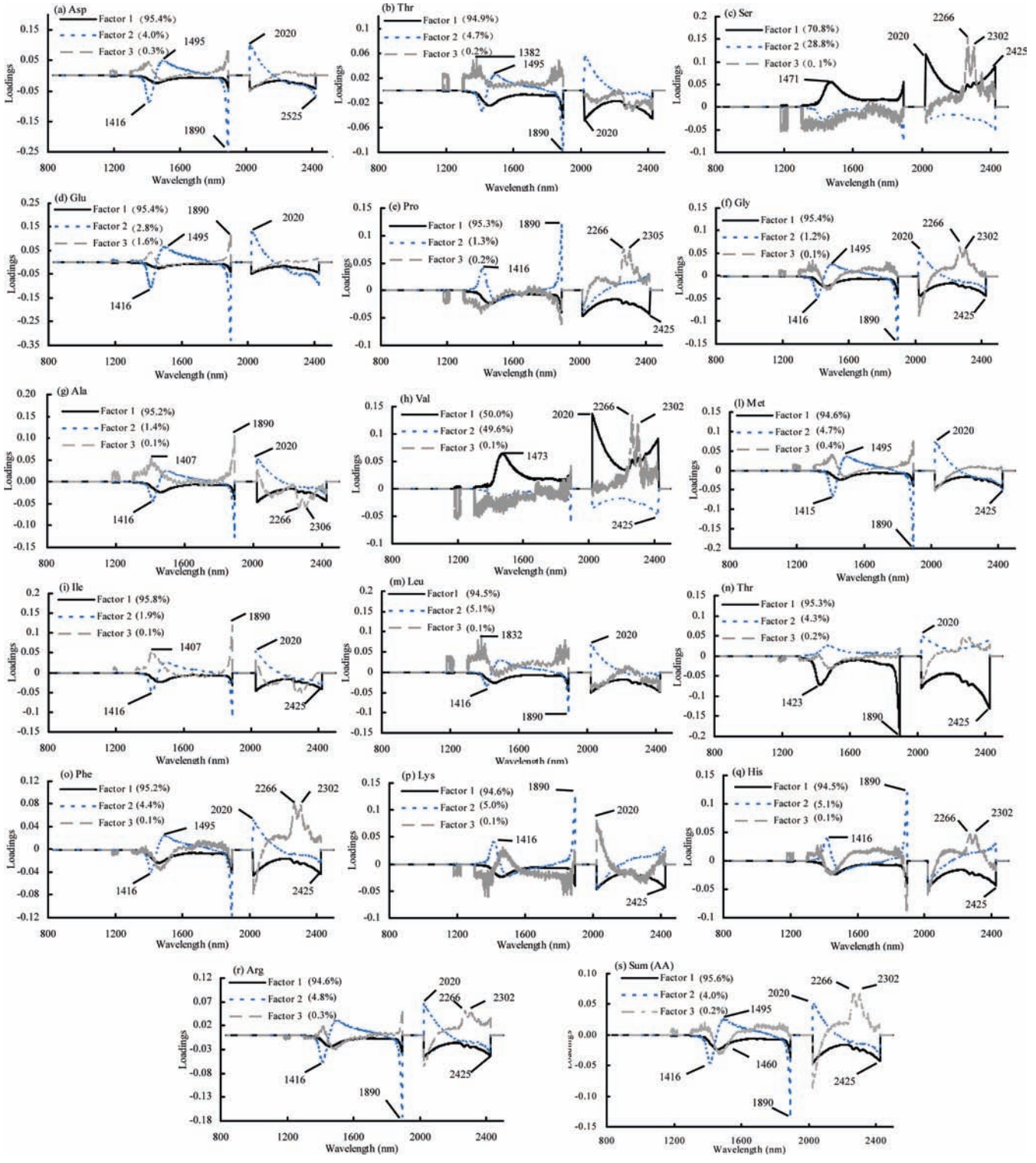

Figure 5. Loading spectra of the first three factors for 16 amino acids and sum (AA) analysis by PLSR.

the calibration for Thr, Ala, Val, Met, Ile, and Tyr gave marked differences between RMSEC and RMSECV (RMSECV > 2RMSEC) and showed a need to improve the precision of the models. For the sum of amino acids (sum (AA)), the RPD and r_{cv} values were higher than 2.0 and 0.84, respectively, which indicated an approximate quantitative prediction. The result indicated that the prediction of amino acids was also dependent on the specific of amino acids. The poor prediction of Pro might relate to the less accurate reference values due to the low response of Pro. The unsatisfactory results obtained for His and Arg might due to the influence of other complex compounds present in rice wines or its structure. Compared to the results obtained by other authors for the determination of amino acids in seeds or feeds, r_{cv} and RPD values obtained for most amino acids in this study were lower than the values in the literature (*36*, *37*), which might due to the relatively small sample set and low concentration of amino acids in Chinese rice wine. However, for the prediction of some amino acids, for example, Ser and Met, r_{cv} and RPD values were better than or close to those in some literature (*38*, *39*). Although the PLSR models need to be improved and validated, the result

obtained in this study suggested that NIR spectroscopy could be used as a tool to determine amino acids in Chinese rice wine rapidly and economically. The HPLC measured values versus FT-NIR predicted values for the 16 amino acids and sum (AA) are shown in **Figure 4**.

PLS Loading Analysis. PLS loading spectra based on the full spectra range were investigated to extract absorption features of amino acids in rice wine samples. The PLS loadings spectra for the first three factors for 16 amino acids and sum (AA) are shown in **Figure 5**. The inverse peaks were found at around 1460 nm of factor 1 for all amino acids except Ser, Val, and Thr, related to O−H overtone in water or carbohydrate. The peaks, inverse or not, found at 1890, 2020, and 2425 nm, constituted the edge of the saturated region, which was reported to be related to O−H stretching and combination of C−H or C−C stretching (*35*). The peaks at around 1423 and 1470 nm in some amino acids were related to O−H or C−H overtone. The highest loading of factor 2 for all amino acids was also found at 1890 nm. The peaks at around 1495 and 1416 nm might be related to first overtone of O−H, N−H stretching, and C−H combination vibrations. The highest loadings of factor 3 found at around 2266 and 2302 nm in some amino acids were mainly related to C−H combinations (*40*−*42*). All of these spectral regions were associated with specific functional groups in amino acids, which helped to explain the basis for the determination of amino acids in Chinese rice wine.

In summary, on the basis of the results of chemometric analyses, NIR spectroscopy might be used as a screening tool to rapidly analyze amino acids in Chinese rice wine without the need for costly and laborious chemical analysis. However, this is just a preliminary study, and further development with larger data sets will be required to improve the precision and robustness of the NIR calibration models.

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