Near-Infrared Rapid Determination of L-Lysine Content in Feed Additive L-Lysine Sulfate (Post-print)

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Abstract

To investigate the feasibility of using near-infrared diffuse reflectance spectroscopy (NIDRS) for rapid quantitative analysis of L-lysine content in feed additive L-lysine sulfate, this study collected 76 representative L-lysine sulfate additive samples nationwide and assigned reference values for L-lysine content using national standard methods. The samples were scanned using a grating-type near-infrared spectrometer to obtain near-infrared spectra under different physical states. Based on L-lysine content, the samples were divided into calibration and validation sets. Employing appropriate spectral preprocessing methods, a near-infrared calibration model for L-lysine sulfate was established using the competitive adaptive reweighted sampling (CARS) algorithm combined with partial least squares (PLS), and this model was compared with a full-wavelength model. The results indicated that the calibration model established using dried, 60-mesh pulverized samples combined with the CARS algorithm was optimal, with a calibration set coefficient of determination (R2C) of 0.954, standard error of calibration (SEC) of 0.510, and standard error of cross-validation (SECV) of 0.659; the validation set coefficient of determination (R2P) was 0.952, the standard error of prediction (SEP) was 0.554, and the ratio of performance to deviation (RPD) value was 3.83. Thus, NIDRS quantitative analysis of L-lysine sulfate demonstrates certain feasibility and holds practical significance for enriching rapid detection methods for amino acid salts and other amino acid products in China.

Full Text

Near Infrared Rapid Determination Method for L-Lysine Content in Feed Additive L-Lysine Sulfate

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Abstract: This study investigated the feasibility of using near infrared diffuse reflectance spectroscopy (NIDRS) for rapid quantitative analysis of L-lysine content in feed additive L-lysine sulfate. Seventy-six representative L-lysine sulfate samples were collected nationwide and chemically analyzed for L-lysine content using national standard methods. Near infrared spectra were acquired for samples in different physical states using a grating-type NIR spectrometer. Based on L-lysine content, samples were divided into calibration and validation sets. A NIR calibration model for L-lysine sulfate was developed using the competitive adaptive reweighted sampling (CARS) algorithm combined with partial least squares (PLS) regression, with appropriate spectral preprocessing, and compared against a full-wavelength model. The optimal calibration model was established using dried, 60-mesh crushed samples with the CARS algorithm, achieving a calibration determination coefficient (R^2C) of 0.954, standard error of calibration (SEC) of 0.510, and standard error of cross-validation (SECV) of 0.659. For the validation set, the prediction determination coefficient (R^2P) was 0.952, standard error of prediction (SEP) was 0.554, and relative percent deviation (RPD) was 3.83. These results demonstrate that NIDRS quantitative analysis of L-lysine sulfate is feasible and holds practical significance for enriching rapid detection methods for amino acid salts and other amino acid products in China.

Keywords: lysine; near infrared diffuse reflectance spectroscopy; rapid determination model; competitive adaptive reweighted sampling

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Introduction

Lysine additives are among the most widely used amino acid additives in animal production, as they not only improve protein utilization efficiency in feed but also enhance feed conversion rates, providing more comprehensive and balanced nutrition for animal growth. In practical applications, free L-lysine is highly hygroscopic, prone to yellowing and deterioration, and has a strong fishy odor. Therefore, except for special requirements, L-lysine is typically pro-

cessed into granular forms such as L-lysine hydrochloride or L-lysine sulfate $[(C_6H_{14}N_2O_2)_2 \cdot nH_2SO_4]$ for use. L-lysine sulfate, developed around 2010, is a novel lysine additive product. The most common commercial specifications contain 65% and 70% L-lysine, with an average content of approximately 55.4%. Compared with L-lysine hydrochloride, L-lysine sulfate offers lower production costs and greater environmental sustainability in post-fermentation processing, although both share the same initial fermentation 工艺. Industrial production typically uses corn starch syrup or molasses as raw materials, undergoes anaerobic fermentation with L-lysine-producing strains, treats the culture broth with sulfuric acid and heat, and finally spray-dries the product into brown granules or powder containing $ $51% L-lysine and no less than 10% other amino acids.

Currently, no unified national or industry standard exists for determining L-lysine content in L-lysine sulfate additives. Most enterprises adopt the acid hydrolysis method from "Determination of Amino Acids in Feed" (GB/T 18246-2000), which, while relatively precise with parallel sample relative deviation <5%, suffers from long hydrolysis times, expensive equipment, high operator skill requirements, and slow analysis speed (approximately 2 days per sample), making it unsuitable for rapid large-batch sample analysis.

Near infrared diffuse reflectance spectroscopy (NIDRS) is an emerging rapid detection technology based on the absorption of near-infrared light by hydrogen-containing groups X-H (such as C-H, N-H, O-H) in molecules. This technique requires no tedious sample pretreatment, uses no chemical reagents, and is pollution-free, making it an environmentally friendly technology with low instrument cost and low detection limits (typically suitable for components >0.1%). NIDRS has been widely applied in feed, petroleum, food, pharmaceuticals, tobacco, tea, and other fields. Since L-lysine is an organic compound and salt formation maintains its stable structure for transport and use, the inorganic components in L-lysine sulfate do not destroy the organic structure, making NIDRS analysis feasible. However, variations in production processes and quality standards among manufacturers result in inconsistent composition, L-lysine content, particle size, and color, while L-lysine sulfate additives are prone to deliquescence, caking, and deterioration during storage and transportation—all factors that influence NIDRS analysis. Although extensive research has applied NIDRS to amino acid detection in food and feed, no studies have reported on amino acid salts and their products.

This study investigated feed-grade L-lysine sulfate additives, analyzing the effects of different sample states on NIR spectroscopic analysis and comparing full-spectrum versus characteristic wavelength calibration models to explore the feasibility of rapid quantitative analysis of L-lysine content using NIDRS.

Materials and Methods

Sample Collection and Preparation

From 2013 to 2016, L-lysine sulfate products with different production dates and specifications were collected in batches from various manufacturers and users, ensuring all samples had distinct origins and strong representativeness. In the first phase, 59 samples from different sources were collected as the calibration set; in the second phase, 17 samples from different sources were collected as the validation set. Each sample was divided into two portions: one portion was crushed using a cyclone mill with a 0.5 mm screen (particle size <0.28 mm, 60 mesh) to prepare ground samples, while the other portion remained as original samples. All samples were sealed and stored at 4°C in a refrigerator.

Determination of L-Lysine Content

L-lysine content was determined using the acid hydrolysis method from "Determination of Amino Acids in Feed" (GB/T 18246-2000) on dried basis samples (dried at 105°C for 5 hours). Ground samples passing through a 60-mesh sieve were weighed (50-100 mg), mixed with 10 mL of 6 mol/L hydrochloric acid solution, vacuum-sealed, and hydrolyzed at 105°C for over 22 hours. After cooling to room temperature, the hydrolysate was filtered, diluted 50-fold, passed through a 0.45 m membrane filter, and analyzed using an amino acid analyzer. Moisture content in as-received basis samples was determined according to "Determination of Moisture in Feed" (GB/T 6435-2014), and L-lysine content in as-received basis samples was calculated based on the dried basis content.

Near Infrared Spectra Acquisition

Spectra were acquired using a SupNIR-2700 grating NIR spectrometer (FPI Instruments) with a wavelength range of 4,000-10,000 cm^{-1}, data sampling interval of 1 nm, and spectral resolution of 10 nm. Since temperature affects sample absorbance and wavelength shift, thereby influencing spectral quality, samples were removed from the refrigerator and equilibrated in the laboratory environment for over 72 hours before scanning to return to room temperature. During sample loading, the height of each sample was leveled with the edge of the sample cell to ensure consistent sample volume. Each sample was scanned three times with repacking, and the average spectrum was obtained as the as-received basis spectrum. All samples were then placed in a 105°C oven for 5 hours, cooled to room temperature, and rescanned following the same procedure to obtain dried basis spectra.

Calibration Model Development

Model Establishment Partial least squares (PLS) regression was used to develop calibration models. PLS simultaneously decomposes both the spectral matrix (X) and concentration matrix (Y). Before calculating each new principal

component, the scores of X (T) and Y (U) are exchanged to maximize correlation between principal components and Y, ensuring optimal calibration models.

Outlier samples were identified using a combination of studentized residuals and leverage values. To eliminate baseline drift and improve spectral resolution, derivative mathematical treatments were applied, expressed as 0-2-21, 1-2-15, 1-2-21, 2-2-15, and 2-2-21, where the first digit indicates derivative order (0=none, 1=first, 2=second), the second indicates polynomial order, and the third indicates derivative gap. To reduce scattering caused by uneven particle size distribution, spectral preprocessing methods including standard normal variate and detrend transformation (SNVDT), standard normal variate (SNV), multiplicative scatter correction (MSC), and detrending (Detrend) were employed.

Model performance was evaluated using an independent validation set, with statistical indicators including: coefficient of determination for calibration (R^2C), cross-validation (R^2V), and prediction (R^2P); standard error of calibration (SEC), cross-validation (SECV), and prediction (SEP); and relative percent deviation (RPD) [ratio of standard deviation (SD) of validation set L-lysine content to SEP]. Generally, smaller SEP and SEC indicate higher model accuracy, while SECV closer to SEC indicates greater model stability.

Characteristic Wavelength Selection Compared with food and feed, L-lysine sulfate additives have relatively simple composition and high L-lysine content, resulting in prominent amino acid-related spectral peaks. To reduce data volume, improve modeling efficiency, and enhance model accuracy and stability, characteristic wavelengths related to L-lysine must be extracted. This study employed the competitive adaptive reweighted sampling (CARS) algorithm to select characteristic wavelengths from the spectra. CARS applies a "survival of the fittest" principle, treating each variable as an individual and using adaptive weighted sampling to retain wavelengths with larger absolute regression coefficients in the PLS model while eliminating those with smaller weights. The algorithm uses cross-validation to optimize the wavelength combination corresponding to the minimum root mean square error of cross-validation. An exponential decay function controls variable retention rates, providing high computational efficiency. All spectral data processing and modeling were performed using MATLAB 2013b (MathWorks, USA) and PLS-Toolbox 8.0 (Eigenvector Research, USA).

Results and Discussion

Representative L-lysine sulfate samples were collected in batches for this study, with statistical results shown in Table 1 . The selected samples exhibited a wide range of L-lysine content with substantial variability, demonstrating strong representativeness.

Near infrared spectra were acquired for L-lysine sulfate samples under four different pretreatment conditions: untreated (as-received basis, not crushed), ground

and dried (60-mesh, dried basis), unground and dried (dried basis, not crushed), and ground but not dried (60-mesh, as-received basis). Various spectral preprocessing method combinations were applied to develop PLS calibration models, with results summarized in Table 2 . The data clearly show that models developed using uncrushed samples performed substantially worse than those using crushed samples, with lower R^2C values and higher SECV and SEC values.

The optimal model was achieved using spectra from samples that were ground to 60 mesh and dried, combined with derivative (2-2-15) and MSC preprocessing, yielding an R^2C of 0.925—significantly superior to models based on other pretreatments. To further improve prediction performance, the CARS algorithm was applied to select characteristic wavelengths related to L-lysine, with parameters including: 5 principal components, 10 cross-validation segments, autoscaling preprocessing, and 50 iterations. The resulting calibration model was compared with the full-spectrum model, with effects of different spectral regions on model performance shown in Table 3 . The CARS algorithm model demonstrated superior prediction performance to the full-spectrum model, with R^2C of 0.954 (higher than 0.925 for full-spectrum) and external validation R^2P of 0.952 (higher than 0.924 for full-spectrum).

Analysis of L-Lysine Content

L-lysine content in samples was measured according to GB/T 18246-2000 and GB/T 6435-2014 methods, with results presented in Table 1. The data indicate that the selected samples covered a broad range of L-lysine content with significant variability, providing strong representativeness for model development.

NIR Spectra of L-Lysine Sulfate

Near infrared spectra of L-lysine sulfate under the four material states are shown in Figure 1 [Figure 1: see original paper]. Although L-lysine sulfate additive is a mixture, its relatively simple composition yields clear and distinct NIR spectral peaks. L-lysine absorption peaks are concentrated in four characteristic spectral regions: the first region (1,050-1,200 nm) corresponds to second overtone C-H group absorption; the second region (1,300-1,500 nm) represents C-H combination band absorption; and the third and fourth regions (1,600-1,850 nm and 2,000-2,502 nm) also exhibit prominent characteristic absorption peaks. At approximately 1,940 nm, absorbance in Figures 1-c and 1-d is significantly lower than in Figures 1-a and 1-b, indicating substantial moisture influence on sample spectra. Compared with uncrushed samples (Figures 1-a and 1-c), crushed samples (Figures 1-b and 1-d) show more uniform particle size, reducing light scattering and resulting in lower absorbance.

Calibration Model Establishment

Based on the physical states of L-lysine sulfate samples, appropriate spectral preprocessing methods (Derivate, SNV, MSC, Detrend, etc.) were selected, and

PLS regression was used to develop NIR calibration models with 59 calibration samples. Results in Table 2 demonstrate that different pretreatments significantly affect model quality. Uncrushed as-received basis samples have large, uneven particles that cause severe light scattering and spectral displacement during scanning, reducing spectral quality and model accuracy.

Moisture content severely impacts sample spectra and directly affects calibration model performance. For 60-mesh as-received basis samples, drying before model development improved R^2C from 0.885 to 0.925 and R^2V from 0.814 to 0.834, demonstrating significant model improvement. Some samples with long storage times experienced varying degrees of deliquescence, affecting model quality. During spectral acquisition, sample temperature had equilibrated to room temperature. Therefore, to obtain high-quality calibration models, samples should be ground and dried before NIR spectral acquisition, with appropriate preprocessing methods applied. The best calibration model achieved R^2C of 0.925, with SEC and SECV of 0.663 and 0.992, respectively, outperforming models based on other material states. However, this model exhibited overfitting, likely due to excessive irrelevant information variables.

Characteristic Wavelength Selection

Developing quantitative models using characteristic wavelengths can effectively eliminate irrelevant variables, improve modeling efficiency, and minimize collinearity effects. In this study, spectra from 60-mesh dried basis samples were preprocessed and analyzed using the CARS algorithm, which selected 31 characteristic wavelengths (Figure 2 [Figure 2: see original paper]). The selected wavelengths correspond well with L-lysine absorption peaks, primarily representing C-H and N-H bond absorptions. The 1,100-1,400 nm region mainly contains second overtone and combination band C-H absorptions, while the 1,700-1,900 nm region is primarily associated with N-H groups.

The CARS-selected wavelengths were used to develop a calibration model evaluated through internal cross-validation and external validation. Internal cross-validation used leave-one-out cross-validation, where one sample was randomly selected from the calibration set (excluded from modeling) to validate the model developed from remaining samples. External validation involved collecting samples from different sources to form an independent validation set for testing the calibration model.

Compared with the full-spectrum model (Table 3), the CARS-PLS model used only 31 wavelengths instead of 1,499, dramatically improving modeling efficiency. Model performance improved significantly, with R^2C increasing from 0.925 to 0.954 and R^2V from 0.834 to 0.924. External validation R^2P increased from 0.924 to 0.952, showing higher correlation between predicted and reference values (Figure 3 [Figure 3: see original paper]). SEC and SECV were smaller with a lower ratio than the full-spectrum model, indicating higher accuracy and better stability. Additionally, eliminating irrelevant variables resolved the over-

fitting issue. The final quantitative analysis model employed: CARS algorithm for wavelength selection, PLS calibration, spectral preprocessing with Derivate (2-2-21) and MSC, achieving SEC of 0.551, SECV of 0.659, and RPD of 3.83.

Using 60-mesh ground and dried samples for NIR spectral acquisition, combined with CARS wavelength selection and PLS modeling, yielded the optimal calibration model. The external validation R^2P of 0.952 demonstrates high accuracy and good stability, confirming the feasibility of this approach for L-lysine sulfate analysis.

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Note: Figure translations are in progress. See original paper for figures.