УДК: 681.7.015.2, 551.501.6, 612.398.192, 614.95

Research article

DOI: 10.32634/0869-8155-2023-377-12-90-94

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Received by the editorial office:
07.11.2023

Accepted in revised:
15.11.2023

Accepted for publication:
01.12.2023

Application of near infrared spectroscopy for identification and quantitative determination of amino acids in their crystalline and salt forms in the preparation of animal feed

ABSTRACT

Methods. For the first time, a comprehensive calibration model has been developed and presented for the rapid determination of basic near-infrared spectroscopy (IR) methods in feed amino acids, with application in the production of animal feed. The research principle is based on the Fourier equation for spectroscopy. In this work, Fourier methods in the Italian region (FTIR, FT-NIRS) were applied. The data obtained from the calibration models were confirmed using high-performance liquid chromatography. FT-NIR predictions agreed well with the chromatography data and had predictive deviation (RPD) values >1.3 in all cases.

Results. The results indicate that FT-NIR spectroscopy can be used as a simple and rapid tool for monitoring amino acids. In the course of the work, experimental confirmation of previously known facts was obtained — the possibility of visual separation of the spectra of not only counterfeit amino acids, but also the possibility of separation by L- and DL-optical isomers. The work shows that the discrepancies between the values obtained by the classical "wet chemistry" method and the values obtained from the constructed calibration models do not exceed the reproducibility limits of arbitration methods.
A predictive model was used based on information flow elements using the OPUS/QUANT2 software package for multivariate calibration and construction of calibration models for amino acids. This chemometric analysis proved the fundamental possibility of determining amino acids in the product. It has been shown that the use of information channels opens up opportunities for the use of many chemometrics algorithms, including data preprocessing and the construction of predictive models.

Key words: infrared spectroscopy, calibrations, amino acids, animal feeding

For citation: N.P. Buryakov *et al.* Application of near infrared spectroscopy for identification and quantitative determination of amino acids in their crystalline and salt forms in the preparation of animal feed. *Agrarian science.* 2023; 377(12): 90–94. https://doi.org/10.32634/0869-8155-2023-377-12-90-94

Научная статья

DOI: 10.32634/0869-8155-2023-377-12-90-94

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Поступила в редакцию:
07.11.2023

Одобрена после рецензирования:
15.11.2023

Принята к публикации:
01.12.2023

Применение инфракрасной спектроскопии ближнего спектра для идентификации и количественного определения аминокислот в их кристаллической и солевой формах при приготовлении кормов для животных

РЕЗЮМЕ

Методы. Впервые разработана и представлена комплексная калибровочная модель для экспресс-определения основного вещества методом инфракрасной спектроскопии в ближней области (ИК) в кормовых аминокислотах, используемых в производстве комбикормов. Принцип исследования основан на уравнении Фурье для спектроскопии. В работе был применен метод Фурье в инфракрасной области (FTIR, FT-NIRS). Данные, полученные с помощью калибровочных моделей, были подтверждены с помощью высокоэффективной жидкостной хроматографии.

Результаты. Прогнозы FT-NIR хорошо согласовались с данными хроматографии и имели значения прогностического отклонения (RPD) >1,3 во всех случаях. Полученные результаты указывают на то, что ИК-спектроскопия FT-NIR может быть использована в качестве простого и быстрого инструмента для контроля аминокислот. В ходе работы были получены экспериментальные подтверждения ранее известных фактов — возможность визуального разделения спектров не только фальсификатов аминокислот, но и возможность разделения по L- и DL-оптическим изомерам. В работе показано, что расхождения между значениями, полученными классическим методом «мокрой химии», и значениями, полученными по построенным калибровочным моделям, не выходят за пределы воспроизводимости арбитражных методов. Была использована прогностическая модель, основанная на элементах информационного потока с применением OPUS/QUANT2 программного пакета для многомерной калибровки и построения калибровочных моделей для аминокислот. Данный хемометрический анализ доказал принципиальную возможность определения аминокислот в продукте.
Показано, что использование информационных каналов открывает возможности для применения многих алгоритмов хемометрики, включая предобработку данных и построение прогностических моделей.

Ключевые слова: инфракрасная спектроскопия, калибровки, чистые формы аминокислот, хемометрия, корма

Для цитирования: Буряков Н.П. *и др.* Применение инфракрасной спектроскопии ближнего спектра для идентификации и количественного определения аминокислот в их кристаллической и солевой формах при приготовлении кормов для животных. *Аграрная наука.* 2023; 377(12): 90–94 (In English). https://doi.org/10.32634/0869-8155-2023-377-12-90-94

Введение/Introduction

The animal industry can be defined as an industry producing proteins of higher value (meat, milk) from less expensive protein sources (vegetable proteins such as soybean meal) [1]. The main function of this dietary protein is to supply amino acids (AA), representing building blocks for polypeptides' synthesis in the animal cells [2]. However, the carbon skeletons of some AA, including L-lysine, L-methionine, L-threonine and L-valine cannot be synthesized from non-AA molecules in cells of any animals. Therefore, they are classified as nutritionally essential AA (EAA) and must be included in diets [3]. Apart from key role of EAA in maintaining physiological functions of cells, tissues and the whole body, they can also have bioactive properties. Particularly, in certain disease conditions they can promote health by improving gut tissue anabolism, reducing stress and modulating immunology [4]. Thus, the adequate dietary supply of nitrogen from protein is of crucial importance to synthesize non-essential amino acids.

Though, the significant part of provided with feed amino components are not used by the animals and excretes in the form of ammonia or nitrate/nitrite leading to the pollution of soil and water [5]. Applying feeding strategies which closely match animal requirements in nitrogen can partly solve this problem. On average, reduction of crude protein content in a diet by one percentage point can yield about an eight to ten percent reduction in nitrogen excretion. Reducing the crude protein level by three to four percent, with supplementation amino acids, can yield the same growth performance but with around 20–30% reduction in nitrogen excretion. Addition of 0.5% L-lysine increases feed quality as much as adding approximately 20% soybean meal [6]. The increase of L-threonine concentration from 0.55 to 0.75% in a corn/sorghum/peanut meal-based diet for young broilers increases the breast meat deposition by more than 15% [1].

Moreover, supplying feed-use amino acids can improve the efficiency of utilization of cultivated areas. In particular, 1 ton of L-Lysine HCl can save the usage of 33 tons of soybean meal, while the arable land required for the production of 48.5 tons of corn, plus 1.5 tons of L-Lysine HCl is about 75% less of that required for 50 tons of soybean meal. Therefore, dietary supplementation of synthetic amino acids may allow nutritionists to further reduce the inclusion of protein rich feedstuffs while maintaining optimum performance and fewer environmental issues from swine and poultry production [2]. However, the variation of raw materials obtained from different suppliers is a common case; even the same supplier can have batch to batch differences, let alone a possibility of falsification. In this regard maintaining the exact amino acid profile of feed demands a constant quality control of incoming materials. Traditional wet chemical analyses such as the high-performance liquid chromatography, allow evaluating amino acids' content from all kind of feed matrices as well as in their pure crystalline forms. Although HPLC is used as a de-facto standard laboratory method, it still has the known disadvantage of a long preparation stage with a number of time-consuming, laborious, and high-cost procedures.

The very promising alternative is the use of Fourier transform near-infrared reflectance spectroscopy (FT-NIR) technique, based on absorption of near-infrared irradiation by the testing sample. This technique allows rapid and nondestructive analysis, representing one of the most suitable approaches for the on-line quality control. While the successful use of FT-NIR as screening tool for monitoring the quality and safety of feed protein materials [7] there are no data about its application for feed used amino acids for the confirmation of claimed content. The main purpose of this study is the development of calibration models that allow the fast and low-cost recognition of amino acids with subsequent determination of essential amino acids' content in their supplied pure crystalline and salt forms.

Материалы и методы исследований / Materials and methods

The research was carried out in 2020–2023 on the basis of the department of feed of the "Cherkizovo" Research Center (Moscow, Russia).

The reference method of determination of main substance' content in amino acids' samples was carried out on HPLC automatic Amino Acid Analyzer S 433 ("Sykam", Germany) with a post-column derivatization and High-Speed Amino Acid Analyzer L-8900 ("Hitachi", Japan) in accordance with ISO 17180[1] (GOST 33428) for lysine sulfate, methionine and threonine and ISO 13903 (GOST 32195)[2] for valine. Samples' spectra were obtained using MPA ("Bruker", Germany) Fourier transform near-infrared spectrometer over the wavelength range from 9000 to 4000 cm^{-1} in the integrating sphere mode at a resolution of 16 cm^{-1}. All the samples were used "as is" in their crystalline powder state supplied by manufactures. Each sample was scanned in a 97 mm cup for 4 times with the repacking after each measurement to minimize any possible influence of components' uneven distribution within the sample. The spectral and wet chemistry data were matched using the OPUSLab (OPUS/LAB — A convenient and intuitive software package for the user to carry out mercury analysis, Bruker (Germany) software to create chemometric models allowing amino acid's type recognition followed by main component quantification. PCA is an unsupervised system directed on identifying patterns in the measured data based on similarity of spectral signature. It is a useful tool for spectral data visualization, allowing an overview of the data set structure by demonstrating the tendency of objects to "aggregate" together based on similarity of spectral features [8].

Object of research: commercial products — feed amino acids: L-lysine, DL-methionine, L-threonine, L-tryptophan and L-valine, L-isoleucine.

Subject of research: detection of concentration and salt form of aminocarboxylic acids. Amino acid samples were collected at the feed production plants of Cherkizovo in accordance with GOST 13496.0[3] and GOST ISO 6497[4] and EN ISO 6497[5].

The mass of the pooled sample (kg) was 1 kg according to EC No. 401/2006[6]. The samples were transported under conditions that ensured the preservation of the condition, composition and quality of the samples, as well as the safety of the environment, on a vehicle equipped for such purposes. The number of samples taken reflected the expected fluctuations within a given batch and was a multiple of 1:10 by the number of bags in the batch. The analysis result did not differ from the actual value by more than 5%.

[1] ISO 17180:2013 Animal feeding stuffs — Determination of lysine, methionine and threonine in commercial amino acid products and premixtures.
[2] ISO 13903:2005 Feeds, compound feeds. Method for determination of amino acids).
[3] GOST 13496.0-2016 Mixed feeding-stuffs. Methods of sampling of average sample.
[4] GOST ISO 6497-2014 Feeding stuffs. Sampling.
[5] EN ISO 6497:2005 No. 76/371/EC "Animal feed - sampling".
[6] Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

The samples were crushed to an acceptable particle size of at least 75 microns.

Feed amino acids from all global suppliers who supplied products to the "Cherkizovo" company (Russia) were selected. These are: "Evonik" (Germany), "Adisseo" (France), "Ajinomoto" (France), "Chael Jedang" (Indonesia), "Bio-Chem" (China).

Product L-Lysine monohydrochloride — 98.5%. Manufacturer: "Sewon" (Korea), CJ (Indonesia), "Ajinomoto" (France), ADM (USA), Belarusian National Biotechnology Corporation — CJSC "BNBK" (Belarus).

Product L-Lysine sulfate — 75%. Manufacturer: "Sewon" (Korea), CJ (Indonesia), Premix Plant No. 1 (Russia), Belarusian National Biotechnology Corporation — CJSC "BNBK" (Belarus).

Product DL-Methionine — not less than 99%. Manufacturer: "Evonik" (Belgium), "Rhodimet" (France), "Sumitomo" (Japan), "Volga methionine" (Russia), "Ningxia Unisplendour Tianhua Methionine (CUC)" and "Shandong NHU Amino Acid (NHU)" (China).

Product L-Threonine — no less than 98.5%. Manufacturer: "Eppen" (China), "Fufeng" (China), "Suihua XMXYG Jingu Biochemical Technology" (China).

Product L-Tryptophan — 98–98.5%. Manufacturer: "Ajinomoto Eurolysine" (France), "PT Cheil Jedang Indonesia" (Indonesia), "Eppen Group" and "Meihua Group" (China).

Product L-Valine — 98%. Manufacturer: "CJ Cheiljedang" (China), "Ajinomoto Eurolysine" (France).

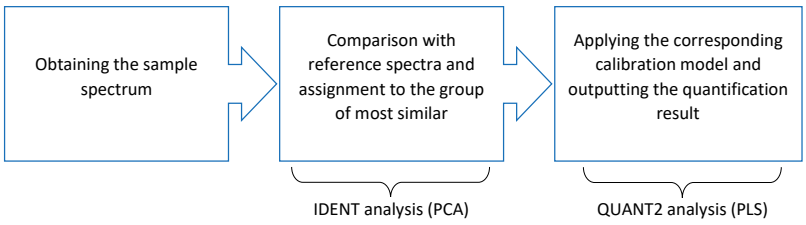
Product L-Isoleucine — 98.5%. Manufacturer: "CJ Cheiljedang" (China), "Fufeng" (China).

At the second step is using chemometric PLS-based method for quantitative analysis[10] with validation procedure according to [11]. For this purpose OPUS QUANT2 software is used. (OPUS/QUANT2 — Software package for multivariate calibration with a huge number of useful graphs, statistics, various tools for building calibration models, automatic selection of tests and removal of unnecessary samples. Bruker (Germany)) the partial least-squares (PLS) regression algorithm [9] was used on spectral data in order to obtain a calibrating model for the quantitative prediction. All the amino acids' calibrations were obtained using the evaluation-set and cross-validation techniques depending on number of available samples.

The scheme of the spectrometric analysis is shown on Figure 1.

For each model, the standard error of calibration (SEC) and correlation coefficients (R^2) were calculated. Moreover, to evaluate the models' predictive value the standard error of prediction (RMSEP) and standard error of evaluation (RMSEE) were also calculated. In addition, the ratio of performance deviation (RPD) was calculated in order to indicate the quality and robustness of a calibration model for each amino acid [6].

Fig. 1. Construction of a chemometric model for combining IDENT and QUANT2 assays



Результаты и обсуждение / Results and discussion

Totally 435 samples of amino acids from different suppliers worldwide were used for the creation of calibration models. Prior to recording spectra, the content of main substance was determined by HPLC method, followed by assigning these values to amino acids' spectra. Then, the principal component analysis was applied to the FT-NIR data matrix as a visualization method for the amino acids' samples distribution giving the 2D-score plot of PC3 vs. PC2 factors (Fig. 1).

Figure 2 indicates excellent separation of different amino acids (L-methionine, L-lysine, L-threonine and L-valine) by their spectral patterns into four easily distinguishable categories without any overlaps, indicating a possibility of unambiguous identification of feed-used amino acids. However, one can see that points tend to group into the subgroups inside each category, especially in methionine case. Such behavior can be explained by the presence of different impurities causing the distortion of spectra and increasing the spectral distance from an average spectrum. Taking into account the fact impurities "fingerprint" is practically unique and depends on used reagents, applied synthesis and purification techniques, this finding provides a potential possibility not only to determine the amino amino acids' type, but also tracing their origin.

To do so, the quantitative determination of the main component content is needed in order to isolate the influence of distortion introduced by impurities. In this regard calibration models resulting from the PLS analysis on correlations between FT-NIR and HPLC measurements on amino acids content were developed. The summary of the performance parameters obtained for the calibration equations is given in the Table 1.

Obtained results showed good agreement between predicted and actual values (Table 1).

From the Table 1 moderate R^2 values can be clearly seen for valine and especially threonine. It can be explained by influence of several factors. Firstly, the high standard deviation (3.07% and 5.78% for valine and threonine respectively) of the reference HPLC method introduces noticeable uncertainty in predicted values of these amino acids content in tested samples. Namely, in threonine case the SD value exceeds the whole tested concentrations range. In other words, even the arbitrary HPLC method can confuse the two samples from the beginning and the end of concentrations range and give the same results. The solution of this problem is enlarging the range of measured concentrations to minimize the influence of measurement uncertainty. However, it leads to second difficulty.

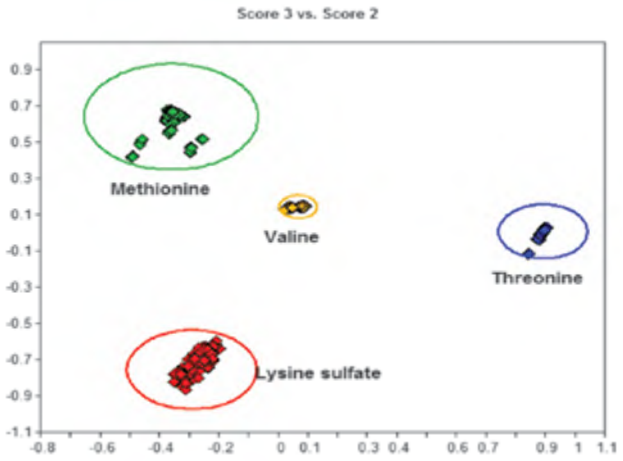
While all the producers declare the 100% amino acid' content in crystalline forms they sell, the real content is

Table 1. **Chemometrics and model parameters for amino acids' calibration set**

Amino acid	Samples number	Concentration range, %		SEC	R^2	RMSEP	RMSEE	SD	RPD
		min	max						
L-Lysine	208	48,52	78,93	0,607	97,78	0,673	0,607	4,07	6,71
L-Methionine	60	94,74	99,91	0,740	97,98	1,191	0,806	2,21	5,56
L-Threonine	96	78,04	99,95	0,587	98,25	0,864	0,586	2,45	6,11
L-Valine	51	93,14	99,73	0,333	93,26	0,513	0,331	1,29	3,87
L-Isoleucine	20	84,61	96,57	0,819	90,74	0,935	0,808	2,69	3,29

*SEC — standard error of calibration, R^2 — correlation coefficient, RMSEP — the models' standard error of prediction, RMSEE — the models' standard error of evaluation, SD — standard deviation, RPD — residual prediction deviation.

Fig. 2. The 2D score plot of amino acids' samples for PC3 and PC2 factors



lower due to impurities. The latter naturally present in different quantity, depending on ways of synthesis and final product purification. Excluding the cases with falsification, the average content of amino acids is almost the same and do not allow the concentrations range enlargement by real samples. At the same time the introduction of artificial mixtures with known second component is also impossible due to significant changes of the amino acid spectrum.

Even the high content of impurities in measured "as is" sample can result in big spectral pattern difference compared to average (Fig. 2).

Nevertheless, obtained chemometric models demonstrate accurate prediction of amino acids' content in their crystalline forms confirming the high robustness and applicability of used calibration equations. Moreover, the prediction error (RMSEP) of the developed calibration model does not exceed reproducibility limits of the reference HPLC method according to ISO 17180 and ISO 13903.

Application of FT-NIR spectroscopy for identification and quantification of amino acids is a convenient and powerful tool with a great potential for rapid routine analysis of incoming feedstuff. The advantage is not only in short processing time but also in a possibility of the quality screening allowing quality improvement and optimum feed formulation by choosing the right raw materials supplier.

Despite the moderate correlation coefficients values for valine and threonine, all the developed calibration models allow FT-NIR determination of amino acids' content with a prediction error lower than reproducibility limits of the reference HPLC method according to ISO 17180 and ISO 13903. Yet, our future work will be directed on further improvement of calibration models by the continuous updating and enlarging samples' data set.

Выводы/Conclusion

The possibility of qualitative recognition with subsequent quantification of 4 essential amino acids (L-methionine, L-lysine, L-threonine and L-valine) by Fourier transform near-infrared (FT-NIR) spectroscopy was shown. The data obtained by partial least-squares (PLS) regression calibration models were confirmed with high-performance liquid chromatography (HPLC) used as a reference method. The FT-NIR predictions were in good agreement with HPLC data and had residual predictive deviation (RPD) values >3.29 in all cases. The obtained results indicate that FT-NIR spectroscopy could be used as a simple, fast and low-cost tool for the control of supplied feed-used amino acids.

Все авторы несут ответственность за свою работу и представленные данные.
Все авторы внесли равный вклад в эту научную работу.
Авторы в равной степени участвовали в написании рукописи и несут равную ответственность за плагиат.
Авторы заявляют об отсутствии конфликта интересов.

All authors bear responsibility for the work and presented data.
All authors have made an equal contribution to this scientific work.
The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.
The authors declare no conflict of interest.

ФИНАНСИРОВАНИЕ

Данное исследование было поддержано Министерством науки и высшего образования Российской Федерации в рамках реализации специальной части гранта Программы стратегического академического лидерства «Приоритет 2030» для развития Российского государственного аграрного университета — МСХА им. К. А. Тимирязева (соглашение № 075-15-2023-220 от 21 февраля 2023 года)."

FUNDING

This research was supported by the Ministry of Science and Higher Education of the Russian Federation as part of the implementation of a special part of the grant of the Strategic Academic Leadership Program "Priority 2030" for the development of the Russian State Agrarian University — named after K. A. Timiryazev (agreement No. 075-15-2023-220 dated February 21, 2023)."

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