Multi-adulterant screening in Gln by ATR-FTIR and advanced chemometrics

Authors: Wenwen Liu, Yu Yin, Shi Li, Yu Yin, Shi Li

Date: 2026-05-22T16:31:49+00:00

Abstract

The rising demand for glutamine supplements has increased the risk of economically motivated adulteration with low-cost substances such as glycine, maltodextrin, and melamine. Conventional detection methods are accurate but time-consuming and unsuitable for rapid screening. This study developed an attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic method combined with chemometrics for simultaneous qualitative and quantitative analysis of multi-adulterant glutamine. Absorption spectra (4000-400 cm-1) of pure glutamine and binary/ternary adulterated mixtures were collected. Soft independent modeling of class analogy (SIMCA), linear discriminant analysis (LDA), and support vector machine (SVM) were used for discrimination, while partial least squares regression (PLSR) and support vector regression (SVR). Characteristic peaks at 1331 cm-1 (glycine), 1023 cm-1 (maltodextrin), and 1558 cm-1 (melamine) served as spectral markers. SVM achieved classification accuracy >0.99 for all adulterants. SVR outperformed PLSR with R2 > 0.99. The platform showed good repeatability. The method is rapid (<2 min/sample), non-destructive, and cost-effective, providing a reliable tool for routine quality control and market surveillance of glutamine supplements.

Full Text

Preamble

Multi-adulterant screening in Gln by ATR-FTIR and advanced chemometrics Wenwen Liu , Yu Yin 1,2,3,* Shi Li 1,2,3,* 1 School of Physics, State Key Laboratory of Integrated Optoelectronics, Northeast Normal University, Changchun 130024, China. 2 School of Physics, Key Laboratory of UV-Emitting Materials and Technology, Northeast Normal University, Changchun 130024, China. 3 College of Physics and Optoelectronic Engineering, Harbin Engineering University, Harbin 150001, China. *Corresponding author:

Abstract

The rising demand for glutamine supplements has increased the risk of economically motivated adulteration with low-cost substances such as glycine, maltodextrin, and melamine. Conventional detection methods are accurate but time-consuming and unsuitable for rapid screening. This study developed an attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic method combined with chemometrics for simultaneous qualitative and quantitative analysis of multi-adulterant glutamine. Absorption spectra (4000-400 cm) of pure glutamine and binary/ternary adulterated mixtures were collected. Soft independent modeling of class analogy (SIMCA), linear discriminant analysis (LDA), and support vector machine (SVM) were used for discrimination, while partial least squares regression (PLSR) and support vector regression (SVR). Characteristic peaks at 1331 cm (glycine), 1023 cm (maltodextrin), and 1558 cm (melamine) served as spectral markers. SVM platform showed good repeatability. The method is rapid (<2 min/sample), non-destructive, and cost- effective, providing a reliable tool for routine quality control and market surveillance of glutamine supplements.

Keywords

ATR-FTIR spectroscopy; Chemometrics; Glutamine; Food adulteration; Multivariate

1. Introduction

Food and drug safety, along with quality regulation, are global concerns. They not only involve the protection of consumer rights but also directly impact human health. Glutamine (Gln) supplements can relieve muscle soreness after intense exercise, boost immunity, and prevent negative symptoms such as fever and colds caused by immune decline during strenuous activity [1-3]. However, the extraction process of Gln is complex and the raw material cost is high, inevitably tempting some manufacturers to cut corners to reduce production costs. In actual food prediction, adulteration methods are becoming increasingly sophisticated. Multiple substances may be added simultaneously to lower costs, or illegal additives may be used to mask off-flavors and colors of low-quality raw materials.

In real-world food testing, adulteration methods have become increasingly sophisticated. To reduce costs, multiple low-cost substances may be added simultaneously. In some cases, illegal additives are used to mask off-flavors or abnormal colors of low-quality raw materials [4-5]. Glycine (Gly), the simplest amino acid, is significantly cheaper than glutamine and has been reported as a common adulterant in amino acid supplements [6]. Maltodextrin (MD), a polysaccharide widely used as a bulking agent, may be added to increase weight or volume at low cost [7-8]. Melamine (MEL), a nitrogen-rich compound, has been notoriously involved in protein-based food fraud incidents due to its ability

to artificially elevate apparent protein content [9-10]. These three adulterants represent different chemical

classes (amino acid, carbohydrate, and nitrogen-rich heterocycle), making them suitable models for developing a broadly applicable detection strategy.

Conventional analytical methods for adulterant detection in supplements include high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis [11-12]. These techniques offer high sensitivity and selectivity but suffer from several drawbacks: they require expensive instrumentation, skilled personnel, time-consuming sample preparation (often involving derivatization), and are not suitable for on-site or rapid screening [13-14].

Therefore, there is an urgent need to develop a rapid, cost-effective, and user-friendly analytical method for routine quality control of glutamine supplements.

Mid-infrared spectroscopy has been widely used for the detection of various complex foods, including honey [15], wheat [16-17], Panax notoginseng powder [18], Tetrahydrocannabinol [19], nanomedicines [20]. Most molecules exhibit strong and unique vibrational absorption peaks in the mid- infrared region (2.5-25 μm), which correspond to different chemical bonds and functional groups, enabling qualitative analysis.

To overcome this limitation, chemometric methods have been widely integrated with spectroscopic techniques for both qualitative and quantitative analysis [21-22]. For classification and discriminant analysis, methods such as Soft Independent Modeling of Class Analogy (SIMCA), Linear Discriminant Analysis (LDA), and Support Vector Machine (SVM) have demonstrated excellent performance in food adulteration detection [23]. For quantitative prediction, Partial Least Squares Regression (PLSR) and Support Vector Regression (SVR) are commonly used to establish robust calibration models [24-25].

The combination of ATR-FTIR with chemometrics has proven effective for single-adulterant detection in various food products, but its application to simultaneous multi-adulterant detection in glutamine supplements remains largely unexplored.

In this study, we developed and validated an ATR-FTIR spectroscopy method combined with multiple chemometric approaches for the rapid, simultaneous qualitative and quantitative analysis of three representative adulterants in glutamine supplements, shown as figure 1 [Figure 1: see original paper]. The specific objectives were: (1) to characterize the MIR spectral features of pure glutamine and each adulterant; (2) to evaluate the performance of SIMCA, LDA, and SVM for discriminating different adulterant types, including two- component and three-component mixtures; (3) to compare PLSR and SVR models for accurate quantification of each adulterant concentration; and (4) to identify robust spectral markers for each adulterant in complex mixtures. The proposed method aims to provide a rapid, cost-effective, and reliable tool for routine

quality control and market surveillance of glutamine supplements.

Flowchart of the detection process for gln adulteration.

2.1 Materials

L-Gln reference standard, Gly, MD, and MEL were all purchased from Sigma Aldrich. To investigate the mid-infrared absorption spectral characteristics of the three adulterants using the ATR- FTIR platform, pure solutions of Gly, MD, and MEL at various concentrations were prepared.

Furthermore, to evaluate the detection efficiency of mid-infrared absorption spectroscopy for multi- adulterant Gln supplements, three types of two-adulterant mixtures and one type of three-adulterant mixture were prepared. The total concentration of all sample solutions was 10 g/L; for example, 50% L- Gln means that the sample solution contained 5 g/L of Gln.

2.2 Spectral

Measurement

Method

Absorption spectra of adulterated Gln samples were collected using a Fourier-transform infrared spectrometer equipped with an ATR accessory and controlled by OPUS 8.7.41 software. The spectral range was 4000-400 cm with a resolution of 4 cm , and the number of scans was set to 32. Before detecting liquid samples, ultrapure water was measured as the background spectrum. All data were preprocessed to remove background noise.

As shown in Table 1 , to evaluate the performance of the ATR-FTIR platform, repeatability predictions were conducted using five different concentrations of pure Gln solutions. The results indicated that the platform achieved an average relative standard deviation of 4.98%, demonstrating good stability.

Repeatability prediction results for L-Gln determination using the ATR-FTIR platform Actual conc.

Mean measured conc. (g/L) SD (g/L) RSD (%) Replicates (g/L)

2.3 Data

Processing Ultrapure water was used as the background spectrum. When measuring sample solutions prepared with ultrapure water, the OPUS software automatically performed spectral subtraction against the water absorption peaks. Before discriminant analysis, centering and scaling were applied. Three discriminant methods, SIMCA, SVM, and LDA, were used for qualitative analysis of the samples. Two regression models, PLSR and SVR, were used for quantitative analysis. The absorption spectral data were divided into a calibration set

(n = 945) and a prediction set (n = 315); the prediction set served as an independent external validation and was not involved in model building. Five-fold cross-validation was performed by randomly splitting the calibration set into five non-overlapping, approximately equal-sized groups. One group was used as the validation set in turn, and the remaining four groups were combined as the calibration set. This process was repeated five times, and the average of the five evaluation results was taken.

Analysis

Adulterants Absorption spectra of the adulterants at different concentrations were collected using the ATR-FTIR platform, and the results are shown in Figure 2 [Figure 2: see original paper]. Gly contains a -COO group, a protonated -NH group,

and a -CH group. This unique molecular structure gives Gly four strong absorption peaks in the mid- infrared region.

The strong absorption peak at 1601 cm is one of the strongest peaks of the ionized -COO group, appearing as a pair with the peak at 1411 cm , representing the antisymmetric and symmetric stretching vibrations of -COO , respectively. The symmetric stretching vibration of -COO involves simultaneous elongation and contraction of the two C-O single bonds and is less intense than the antisymmetric vibration. However, the peak at 1411 cm is not only due to -COO alone; the bending vibration of -CH also appears in this region, and the two may couple. The in-plane rocking vibration of -CH arises from the back-and-forth oscillation of two hydrogen atoms in the molecular plane. This vibrational mode is significantly enhanced in Gly by interaction with the strongly polar -NH and -COO groups, giving the characteristic peak at 1331 cm . In addition, the peak at 1510 cm corresponds to the symmetric bending vibration of -NH in Gly. As shown in Figure 2, the sensitivities at 1601 cm and 1331 cm are 7.27 × a.u./(g/L) and 5.32 × 10 a.u./(g/L), with coefficients of determination (R) of 0.99977 and 0.9999, respectively.

Mid-infrared absorption spectra of Gly and corresponding sensitivities. (a) Absorption spectra; (b) calibration curves of peak intensity at 1601 cm and 1331 cm versus concentration.

Mid-infrared absorption spectra of MD and corresponding sensitivities. (a) Absorption spectra; (b) calibration curves of peak intensity at 1153 cm and 1023 cm versus concentration.

MD is a polysaccharide composed of short-chain polymers of glucose units linked by glycosidic bonds. The MD used in this study has a dextrose equivalent (DE) of 13-17, which is the most common type used as a food additive. In the structure of MD, each glucose unit contains multiple C-O bonds, including C-O-C of glycosidic bonds and C-O-H on the ring. The stretching vibrations of these C-O bonds give rise to the characteristic peaks in the absorption spectrum.

The peak at 1023 cm is one of the strongest and most characteristic peaks of polysaccharides; it represents the symmetric stretching

vibration of C-O on the sugar ring and the stretching vibration of C-C, which is the overall skeletal vibration of the sugar ring. The peak at 1153 cm directly reflects the glycosidic bond skeleton connecting glucose units. The C-O-C group of the glycosidic bond exhibits a strong characteristic absorption in this region, providing direct evidence of the presence of polysaccharide chains. As shown in Figure 3 [Figure 3: see original paper], the absorption intensities at 1023 cm and 1153 cm increase steadily with increasing MD concentration. Their sensitivities are 5.34 × 10 a.u./(g/L) and 2.17 × 10 a.u./(g/L), with R values of 0.99904 and 0.99829, respectively, demonstrating that the ATR-FTIR platform enables high-precision quantitative analysis of MD.

MEL has the molecular formula C . Its core is a symmetric 1,3,5-triazine ring with three carbon atoms each bonded to an amino group. Therefore, the infrared absorption spectrum of MEL is mainly derived from the vibrations of the triazine ring skeleton and the amino groups attached to the ring. In the range of 1800-800 cm , the mid-infrared absorption spectrum of MEL shows three characteristic peaks at 1463 cm , 1558 cm , and 1624 cm . Among them, the peak at 1558 cm exhibits the strongest absorption intensity and can serve as the primary observation peak. The peak at 1624 cm corresponds to the stretching vibration of the C N bonds in the triazine ring and may also involve in-plane bending vibrations of N-H. This peak provides strong evidence for the existence of the conjugated triazine ring, where the C N bond order is between single and double bonds, and the stretching frequency of such a conjugated system is lower than that of an isolated C=N double bond. Furthermore, the ring breathing vibration and the ring deformation vibration of the triazine ring appear at 1463 cm and 1558 cm respectively. As shown in Figure 4 [Figure 4: see original paper], the sensitivities at these two peaks are 8.28 × 10 a.u./(g/L) and 0.00176 a.u./(g/L), with R values of 0.99803 and 0.99917, respectively. The ring breathing vibration is a symmetric ring stretching vibration in which all bonds in the ring stretch and contract almost simultaneously and in phase, causing the entire ring to "breathe" . The ring deformation vibration is an asymmetric ring stretching vibration in which different bonds in the ring stretch asynchronously; this mode has lower symmetry and can easily couple with vibrations of the amino groups attached to the ring.

Mid-infrared absorption spectra of MEL and corresponding sensitivities. (a) Absorption spectra; (b) calibration curves of peak intensity at 1558 cm and 1463 cm versus concentration.

Analysis

Adulterated Multiple Substances L-Gln reference standards at various concentrations were measured using the ATR-FTIR platform, and the results are shown in Figure 5 [Figure 5: see original paper]. Compared with the tapered fiber

FTIR platform, the mid-infrared absorption spectra of pure Gln obtained by the ATR-FTIR platform showed minor differences, mainly in the positions and intensities of the characteristic peaks.

As shown in Figure 5, regarding the shift in characteristic peak positions, the Gln absorption spectra collected by this platform still exhibit four main characteristic peaks: 1666 cm , 1605 cm , 1518 cm

and 1408 cm . The peak at 1666 cm is the most typical infrared absorption peak of the amide group, usually appearing in the range of 1680-1630 cm , corresponding to C=O stretching with minor coupling from C-N and N-H bending. The peak at 1518 cm arises from the coupled vibration of N-H bending and C-H stretching. Notably, the peak at 1605 cm originates not only from the antisymmetric stretching vibration of -COO but may also be superimposed with the bending vibration of -NH . Under this platform, the peak at 1605 cm exhibits higher absorption intensity, mainly because the direction of the evanescent wave electric field from the ATR crystal matches the vibrational dipole moment of -NH the surface environment, facilitating the formation of hydrogen bonds and increasing the local concentration of -NH groups. In contrast, the peak at 1408 cm is influenced solely by the symmetric stretching vibration of -COO . Therefore, the absorption intensity in this range better reflects the sensitivity difference between the tapered fiber FTIR and ATR-FTIR platforms. The only peak that does not shift is at 1348 cm , which is assigned to the C-H bending vibration of -CH (a) Mid-infrared absorption spectrum of Gln; (b) calibration curves of peak intensity at 1605 cm and 1408 cm versus concentration.

Figures 6-9 show the mid-infrared absorption spectra and the corresponding calibration curves at the characteristic peaks for the four types of multi-adulterant mixtures. Owing to the unique structures of the three adulterants - the -CH group of Gly, the glycosidic bond of MD, and the triazine ring of MEL - the vibrations of these structural units appear at 1331 cm , 1023 cm , and 1558 cm , respectively, in the mid-infrared absorption spectra of the multi-adulterant mixtures. Therefore, these three peaks can serve as key observational peaks for quantitative analysis of adulteration levels.

In multi-component mixed solutions, the characteristic peaks of different substances may overlap, interfere, or differ in intensity. However, the peak at 1331 cm clearly points to the -CH backbone of Gly. The -CH group is relatively hydrophobic and nonpolar, and its vibration is less affected by the external polar environment. When the hydrogen-bonding network is reorganized, its vibration frequency and intensity remain relatively constant. Moreover, the side chain of Gln is -CH , and its bending vibration occurs near 1350 cm , which is different from the backbone environment. MEL is an aromatic heterocycle without aliphatic -CH . MD has C-H vibrations mainly at higher wavenumbers and other positions in the fingerprint region. Therefore, the peak at 1331 cm can serve as a quantitative marker for Gly.

In the absorption spectra of multi-component mixed samples, the peak at 1023

cm is unique to MD. The molecular structures of the other three substances do not contain the long-chain structure of repeating glucose units connected by α-1,4 glycosidic bonds found in MD. The glycosidic bond lies between two glucose rings, and its vibration depends on the conformation of the glucose rings rather than being directly exposed to intermolecular interactions. Thus, it is less sensitive to external interference than the vibrations of exposed O-H groups. Furthermore, the characteristic peaks arising from C-O or C-

N vibrations of the other three substances are far from this peak position, so even in the presence of interfering substances, this peak remains relatively stable.

Adulterant type Gln-Gly-MD-MEL. (a) Mid-infrared absorption spectra; (b) calibration curve at the Gly characteristic peak of 1331 cm ; (c) calibration curve at the MD characteristic peak of 1023 cm ; (d) calibration curve at the MEL characteristic peak of 1558 cm .For the adulterant type Gln-Gly-MD-MEL, the notation 50-5-5-40 means that in a 10 g/L mixed solution, it contains 5 g/L Gln, 0.5 g/L Gly, 0.5 g/L MD, and 4 g/L MEL.

Adulterant type Gln-Gly-MD. (a) Mid-infrared absorption spectra; (b) calibration curve at the Gly characteristic peak of 1331 cm ; (c) calibration curve at the MD characteristic peak of 1023 cm . For the adulterant type Gln-Gly-MD, the notation 50-5-45-0 means that in a 10 g/L mixed solution, it contains 5 g/L Gln, 0.5 g/L Gly, and 4.5 g/L MD, with no MEL.

In addition, the peak at 1558 cm reflects the core vibration of the rigid aromatic ring of MEL and possesses high structural specificity. Its absorption intensity is strong and not easily masked or assimilated by non-cyclic vibrations of other substances. Owing to electron delocalization and structural rigidity, the skeleton vibration force constant of the aromatic ring is large, and its frequency is very fixed.

Even if the nitrogen atoms on the ring participate in hydrogen bonding, the effect on the overall electron

distribution and rigidity is limited and insufficient to cause a large shift in this skeletal vibration frequency. The peaks of Gly and Gln in this region belong to the amide II band or -NH bending vibrations, which originate from completely different functional groups and vibrational modes. Although their positions are close, the nature of the vibrations is different. Therefore, this characteristic peak can still serve as a marker for MEL quantification.

Adulterant type Gln-Gly-MEL. (a) Mid-infrared absorption spectra; (b) calibration curve at the Gly characteristic peak of 1331 cm ; (c) calibration curve at the MEL characteristic peak of 1558 cm . For the adulterant type Gln-Gly-MEL, the notation 50-5-0-45 means that in a 10 g/L mixed solution, it contains 5 g/L Gln, 0.5 g/L Gly, and 4.5 g/L MEL, with no MD.

Gln-Gly-MD Gln-Gly-MEL Gln-MD-MEL Gln-Gly-MD-MEL

The sensitivities and coefficients of determination for the characteristic peaks

of different adulterant types are listed in Table 2 . Overall, the R values for most characteristic peaks are above 0.97, indicating a strong linear relationship between the selected peak intensities and the adulterant concentrations, providing good potential for quantitative analysis. The peak at 1558 cm shows relatively high sensitivity in all adulteration systems containing MEL, with the best performance observed in the Gln-MD-MEL system (1.94 × 10 a.u./wt%). In contrast, the R values for the 1023 cm peak are generally slightly lower, reflecting a somewhat weaker linear correlation. Although the change in absorption intensity at 1023 cm can reflect the relative content of MD, it cannot be directly used for accurate quantification; thus, high-precision chemometric methods are required for quantitative analysis of the samples.

Adulterant type Gln-MD-MEL. (a) Mid-infrared absorption spectra; (b) calibration curve at the MD characteristic peak of 1023 cm ; (c) calibration curve at the MEL characteristic peak of 1558 cm . For the adulterant type Gln-MD-MEL, the notation 50-0-5-45 means that in a 10 g/L mixed solution, it contains 5 g/L Gln, 0.5 g/L MD, and 4.5 g/L MEL, with no Gly.

Analysis

The above qualitative analysis based on characteristic peaks can only achieve preliminary identification of substance types; it is difficult to accurately discriminate the adulterant categories and adulteration levels of Gln-adulterated samples, and it is susceptible to baseline drift, noise interference, and peak overlap. To achieve rapid and accurate discrimination of adulterated Gln samples, three chemometric methods (LDA, SIMCA, and SVM) were further introduced. Discriminant models were constructed based on the mid-infrared absorption spectra of adulterated samples, and their discriminative performances were compared to select the optimal model, providing a qualitative discrimination basis for the adulteration detection of Gln supplements.

SIMCA was used to discriminate the adulterant types, enabling classification of unknown samples into adulterant categories. Table 3 shows the inter-class distances for various adulterant types in Gln.

The inter-class distance is a comprehensive index used to evaluate the degree of separation between two different classes in the SIMCA model. In this study, the inter-class distances for all four adulterant types were far greater than 3, indicating that the SIMCA model is ideal. axis represents the distance of a sample to the PCA-Gln-Gly-MD model, and the y-axis represents the distance to the PCA-Gln-Gly-MEL model. The vertical/horizontal reference lines represent the critical

distance lines; points to the right/above these lines indicate that the sample distance exceeds the acceptable normal range for that class. The critical distance lines divide the Cooman' s plot into four regions: the upper-left region represents samples belonging to class Gln-Gly-MD; the lower-right region represents samples belonging to class Gln-Gly-MEL; the upper-right region is the

intermediate region, where samples cannot be clearly assigned to either class but lie within the joint acceptance domain of both models; the lower-left region is the outlier region, where samples lie outside the joint acceptance domain. The Cooman' s plots show that the classes are effectively separated. Table 4 presents the discriminant results of the SIMCA analysis. Model performance was evaluated using four metrics: accuracy, precision, recall, and F1 score. For the multi-adulterant discrimination, the performances for Gln-Gly-MD, Gln-Gly-MEL, and Gln-MD-MEL were relatively better, with precision reaching 1 for both calibration and prediction sets, and accuracy and recall both above 0.9. This indicates that the SIMCA model fits these three adulterant types well and exhibits good generalization ability for unknown samples, with overall stable and reliable discriminant performance.

Inter-class distances for four multi-adulterant types in SIMCA PCA-Gln-Gly- PCA-Gln-Gly- PCA-Gln-MD- PCA-Gln-Gly- MD-MEL Cooman' s plots of SIMCA analysis. (a) Classification of Gln-Gly system in the presence of MD and MEL; (b) classification of Gln-MD system in the presence of Gly and MEL; (c) classification of Gln-MEL system in the presence of Gly and MD. The threshold for all plots is 5%.

However, for the four-component mixed adulterant type Gln-Gly-MD-MEL, the model' s discriminant performance decreased. The main reason is that this four-component mixture contains component subsets that overlap with the previous three adulterant types, leading to significant overlap between its model and those of the three-component types. Consequently, samples originally belonging to the three-component types can be easily misclassified as the four-component type. Nevertheless, it is noteworthy that the recall for this type remained above 0.92, indicating that although the model' s recognition ability for multi-component mixed adulteration still has room for improvement, it can still effectively detect adulterated samples and avoid severe false negatives.

SIMCA discriminant results for the four multi-adulterant types Gln-Gly-MD Gln-Gly-MEL Gln-MD-MEL Gln-Gly-MD-MEL In summary, the SIMCA model exhibits excellent discriminant performance for two-component adulterated Gln samples. Its discriminative ability is slightly reduced only for adulterant types with component inclusion relationships, but overall, the model can provide reliable technical support for the detection of multi-adulterant Gln.

LDA and SVM were used to discriminate the presence of specific components in the samples; these methods can determine whether a sample contains a given component. The discriminant results of the LDA model are shown in Table 5 . For Gly adulteration, all evaluation metrics were above 0.93, and the F1 score reached 0.96, indicating that the LDA model has excellent comprehensive classification performance, accurately identifying adulterated samples while avoiding misclassifications. For MD adulteration, all metrics were 1, demonstrating ideal discriminant ability, completely distinguishing samples without MD from those with MD. For MEL adulteration, the LDA model also performed very well, with accuracy, precision, recall, and F1 scores all above 0.97 for both calibration and

prediction sets, indicating excellent fitting and generalization ability.

Figures 11-13 show the linear discriminant plots of the LDA model for the three adulterants, visually presenting the separation between authentic and adulterated samples in the LDA feature space. Taking the linear discriminant plot for Gly as an example, the axes represent the feature projection directions corresponding to the LDA discriminant functions, and the numerical values are the projections of the sample spectral data onto the two discriminant directions. Blue points represent authentic samples without Gly, and pink points represent adulterated samples containing Gly. The two classes are clearly

separated in the feature space, further verifying the good discriminant ability of the LDA model for Gly adulteration. The LDA model effectively makes samples of the same class more clustered and samples of different classes more separated, enabling linear separation. The small amount of overlap reflects the presence of low-concentration adulteration or spectrally similar samples.

LDA discriminant results for Gly, MD, and MEL in multi-adulterant samples Scatter plots of LDA feature space distribution for authentic samples vs. Gly-adulterated samples. (a) Calibration set; (b) prediction set. Blue: samples without Gly; pink: samples adulterated with Gly.

Scatter plots of LDA feature space distribution for authentic samples vs. MD-adulterated samples. (a) Calibration set; (b) prediction set. Green: samples without MD; orange: samples adulterated with MD.

Scatter plots of LDA feature space distribution for authentic samples vs. MEL-adulterated samples. (a) Calibration set; (b) prediction set. Purple: samples without MEL; red: samples adulterated with MEL.

The separation effects in Figures 10-12 also reflect the four evaluation metrics. Among the three scatter plots, the separation for MD adulteration is the most obvious, with the highest accuracy, precision, recall, and F1 score; MEL is next. In Figure 10 [Figure 10: see original paper] (Gly), the overlap between the two classes is the largest, and accordingly its metrics are the lowest.

In this study, the SVM model used an RBF kernel and the C-SVC type. The penalty coefficient $C = 1$, and the kernel parameter $\gamma = 0.001422475$. The discriminant results of SVM are shown in Table 6 .

For the discrimination of single adulterants (Gly, MD, or MEL) in Gln, the SVM method achieved extremely high precision. In particular, the recognition of MD and MEL reached perfect accuracy, with all parameters equal to 1. This indicates that the SVM model can effectively find the optimal classification hyperplane for discriminating whether a given component is present in multi-adulterant Gln supplements, enabling accurate classification.

SVM discriminant results for Gly, MD, and MEL in multi-adulterant samples Calibration Prediction Calibration Prediction Calibration Prediction In summary, among the three discriminant methods, SVM performed the best for

multi-adulterant discrimination of Gln. SVM demonstrates its unique advantage in handling nonlinear data in such complex mixed solutions.

Analysis

Market supervision requires not only the identification of adulteration but also the determination of the specific content of adulterants. Therefore, based on the successful construction of qualitative

discriminant models, this study further conducted quantitative analysis. Using mid-infrared absorption spectral data, quantitative models such as PLSR and SVR were introduced to establish the relationship between spectral features and adulterant concentrations. Under the condition of simultaneous adulteration with multiple substances, rapid quantitative prediction of adulterant contents in Gln samples was achieved, providing data support for assessing adulteration levels and establishing regulatory standards.

PLSR results for multi-adulterant samples. (a) Gly; (b) MD; (c) MEL.

Regression results for multi-adulterant samples Calibration Set Validation Set Prediction Set Model Adulterant SVR results for multi-adulterant samples. (a) Gly; (b) MD; (c) MEL.

The quantitative models were evaluated using RMSEC, RMSECV, RMSEP, and R for calibration, validation, and prediction sets. The regression results are shown in Table 7 and Figures 14-15. Both regression models performed well. Notably, SVR, leveraging the unique advantages of SVM in nonlinear

thinking, performed better in complex matrix systems, with R values for calibration, validation, and prediction sets all greater than 0.99, and root mean square errors within reasonable ranges.

4. Conclusion

This study investigated the qualitative and quantitative analysis of multi-adulterant Gln using an ATR-FTIR platform combined with chemometric methods including SIMCA, SVM, LDA, PLSR, and SVR. The spectral regions most affected by the adulterants in pure Gln are the -CH bending vibration of Gly, the C-O stretching and C-C stretching vibrations of the sugar ring in MD, and the triazine ring stretching vibration of MEL. The characteristic peaks at 1331 cm (Gly), 1023 cm (MD), and 1558 cm (MEL) were identified as spectral markers. The results of the SIMCA, SVM, and LDA models showed that discriminant models can accurately perform qualitative analysis for multi-adulterant types. Among them, the SVM model achieved the best performance, with an accuracy of above 0.99. The results of the PLSR and SVR regression models indicated that for the quantification of the three adulterants, the SVR model exhibited R values greater than 0.99, outperforming the PLSR

model, demonstrating that the SVR model can accurately quantify adulterant contents after discriminant identification.

Acknowledgements This work is supported by the National Natural Science Foundation of China (NSFC) under Grants No. 62090064, 62225502, 62105079, and 62205085; The Fundamental Research Funds for the Central University (No. 3072024XX2513); Natural Science Foundation of Heilongjiang Province (No.

KY12500250008); The National Key Research and Development Program of China (No. 2020YFA0607602).

CRediT authorship contribution statement Wenwen Liu : Writing-original draft, Methodology, Investigation, Formal analysis.

Yu Yin : Writing- review & editing, Conceptualization, Supervision, Formal analysis.

Shi Li : Supervision, Resources, Conceptualization.

References

P. R. Espeño, A. K. S. Ong, J. D. German, et al., "Analysis of Actual Fitness Supplement Consumption among Health and Fitness Enthusiasts," Foods , 21 (2024).

A. V. Caris and R. V. Thomatieli-Santos, "Carbohydrate and Glutamine Supplementation Attenuates the Increase in Rating of Perceived Exertion during Intense Exercise in Hypoxia Similar to 4200 m," Nutrients , 10 (2020).

A. Martín-Rodríguez, P. Belinchón-deMiguel, A. Rubio-Zarapuz, et al., "Advances in Understanding the Interplay between Dietary Practices, Body Composition, and Sports Performance in Athletes," Nutrients , 32 (2024).

H. F. Li, H. L. Mo, Y. C. Song, et al., "The integration of machine learning into proteomics advances food authentication and adulteration control," Trends Food Sci. Technol. , 11 (2025).

C. Tan, B. Cheng, M. X. Chen, et al., "Feasibility of ATR-MIR spectroscopy to detect and to quantify adulterants in whey protein concentrate powder," J. Food Compos. Anal. , 8 (2025) T. Nuzzo, A. Mancini, M. Miroballo, et al., "High performance liquid chromatography determination of L-glutamate, L-glutamine and glycine content in brain, cerebrospinal fluid and blood serum of patients affected by Alzheimer' s disease," Amino Acids , 435-449 (2021).

X. Z. Jiao, Y. Y. Meng, K. K. Wang, et al., "Rapid Detection of Adulterants in Whey Protein Supplement by Raman Spectroscopy Combined with Multivariate Analysis," Molecules , 13 (2019).

M. S. Martins, M. H. Nascimento, L. L. Barbosa, et al., "Detection and quantification using ATR-FTIR spectroscopy of whey protein concentrate adulteration

with wheat flour," LWT-Food Sci. Technol. 6 (2022).

U. Sushma, A. K. Srivastava, M. H. Krishnan, "Melamine Detection in Food matrices employing Chicken Antibody (IgY): A Comparison between Colorimetric and Chemiluminescent Methods," 15, 668-677 (2019).

J. Sun, X. Zhang, Y. Cao, et al., "Ovarian Toxicity in Female Rats after Oral Administration of Melamine or Melamine and Cyanuric Acid," PLoS ONE, 11, e0149063 (2016).

D. Montesano, O. Gennari, C. Festa, et al., "A Simple HPLC-DAD Method for the Analysis of Melamine in Protein Supplements: Validation Using the Accuracy Profiles," J. Chem. , 7 (2013).

V. R. Kozhuharov, K. Ivanov, D. Karcheva-Bahchevanska, et al., "Development and Validation of Gas Chromatography-Mass Spectrometry Method for Quantification of Sibutramine in Dietary Supplements," Processes , 18 (2023).

M. S. Martins, M. H. Nascimento, L. L. Barbosa, et al., "Detection and quantification using ATR-FTIR spectroscopy of whey protein concentrate adulteration with wheat flour," LWT-Food Sci. Technol. 6 (2022).

R. Sethuraman, T. L. Lee, and S. Tachibana, "Simple quantitative HPLC method for measuring physiologic amino acids in cerebrospinal fluid without pretreatment," Clin. Chem. , 665-669 (2004).

K. Ruoff, M. T. Iglesias, W. Luginbühl, et al., "Quantitative analysis of physical and chemical measurands in honey by mid-infrared spectrometry," Eur. Food Res. Technol. 223, 22-29 (2006).

S. Sharma, K. N. Uttam, "Nondestructive and rapid probing of biochemical response of arsenic stress on the leaves of wheat seedlings using attenuated total reflectance fourier transform infrared spectroscopy," Anal. Lett. 52, 268-287 (2019).

S. Freitag, M. Anlanger, P. Fomina, et al., "Attenuated total reflection mid-infrared spectroscopy to screen Austrian and French wheat from multiple years for deoxynivalenol," Spectrochim. Acta A Mol.

Biomol. Spectrosc. (2025).

F. Ma, J. Chen, X. Wu, et al., "Rapid discrimination of Panax notogeinseng of different grades by FT-IR and 2DCOS-IR," J. Mol. Struct. 1124, 131-137 (2016).

N. Geskovski, G. Stefkov, O. Gigopulu, et al., "Mid-infrared spectroscopy as process analytical technology tool for estimation of THC and CBD content in Cannabis flowers and extracts," Spectrochim.

Acta A Mol. Biomol. Spectrosc. 251, 119422 (2021).

G. Carissimi, M. G. Montalbán, G. Víllora, et al., "Direct quantification of drug loading content in polymeric nanoparticles by infrared spectroscopy," Pharmaceutics 12, 912 (2020).

M. F. Dupont, A. Elbourne, D. Cozzolino, et al., "Chemometrics for environmental monitoring: a review," Anal. Methods (202X).

M. Bevilacqua, R. Bro, F. Marini, et al., "Recent chemometrics advances for foodomics," TrAC Trends Anal. Chem. 96, 42-51 (2017).

S. Zhao, B. Zhang, J. Yang, et al., "Linear discriminant analysis,"Nature Reviews Methods Primers 70 (2024).

R. Xu, M. Z. Adil, S. Jabeen, et al., "Recent advancements in chemometrics based non-destructive analytical techniques for rapid detection of adulterants in milk and dairy products - A review," Food Control , 20 (2025).

Z. Li, X. Wang, and U. Kruger, "Efficient cross-validatory algorithm for identifying dynamic nonlinear process models," Control Eng. Practice , 17 (2021).

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