

Dear reviewer

Thank you very much for reviewing our manuscript. We would also like to express our gratitude to the reviewers for their efforts in helping to improve our manuscript titled "Stacked BiLSTM-Adaboost Collaborative Model: Construction of a Precision Analysis Model for GABA and Vitamin B9 in the foxtail millet" (ID: agronomy-3781699). The comments were all valuable and very helpful for revising and improving our paper. Our research team have studied each reviewers' comments point by point and have made necessary modifications and supplements, which we hope will meet with your approval. Revised portion are mark by "Track Change" in bold font throughout the revised manuscript with track change (Please download the file "Revised Manuscript with track change"), and the point-by-point responses are listed as follows.

Abstract: It is recommended to clearly describe the objective of the work after introducing the topic. It is recommended to describe the design and treatments adopted. The conclusion should be based on the results. The conclusion should be related to the work.

Amidst the health-conscious consumption trend, functional foods rich in γ -aminobutyric acid (GABA) and vitamin B9 are gaining prominence. Foxtail millet, a traditional grain naturally abundant in these nutrients, faces quality assessment challenges due to the time-consuming and destructive nature of conventional methods, hindering large-scale screening. This study pioneers the systematic application of hyperspectral imaging (HSI) for non-destructive detection of GABA and vitamin B9 in millet. Utilizing spectral data from 190 samples across 19 varieties, we developed an innovative "coarse-fine" feature wavelength selection strategy. First, interval-based algorithms (iRF, iVISSA) screened highly correlated wavelength subsets. Second, model population analysis (MPA) algorithms (CARS, BOSS) identified optimal core wavelengths, boosting model efficiency and robustness. Based on this, a stacked BiLSTM-Adaboost model was built, integrating bidirectional long short-term memory networks for sequence dependency and adaptive boosting for enhanced generalization. This enables efficient, rapid, non-destructive, and precise nutrient detection. This interdisciplinary breakthrough establishes a novel pathway for millet nutritional assessment, deepens fundamental research, and provides core support for industrial upgrading, breeding, quality control, and functional food development, supporting national health.

Keywords should be different from the words in the title.

Keywords: Foxtail millet; Hyperspectral imaging; γ -aminobutyric acid; Vitamin B9; Nondestructive detection

Introduction: It is recommended to remove Figure 1 from the introduction. The introduction should follow the "inverted pyramid" model, starting with general information about the topic and gradually specifying it, concluding with the objective of the work.

we have removed Figure 1 from the manuscript.

In line 51-67: GABA is a non-protein amino acid that is widely distributed in

microorganisms, plants, and animals. It offers a range of nutritional benefits. Notably, the GABA content in foxtail millet can reach 20 - 50 mg/100g, which is markedly higher than that found in common grains [11]. This relatively high GABA level makes foxtail millet a valuable source of this important nutrient. Vitamin B9 is an essential vitamin for the human body. It is involved in numerous biochemical reactions and is vital for maintaining normal physiological functions. Vitamin B9 content in foxtail millet ranges from 30 - 50 μ g/100g, which is higher than that in staple crops like corn and wheat [12]. This higher concentration of vitamin B9 in foxtail millet enhances its nutritional value compared to other commonly consumed grains. Given the superior content of both GABA and vitamin B9 in foxtail millet relative to other crops, achieving rapid and non-destructive detection of these two nutrients holds high practical significance. Traditional chemical detection methods, however, have drawbacks such as long detection cycles, high costs, and sample destruction, making it challenging to meet the demands of modern food nutrition analysis. Therefore, developing a rapid, accurate, and non-destructive analysis technology for GABA and vitamin B9 in foxtail millet is of great importance.

In line 91-114: At present, with the rapid development of computer science, the fusion of deep learning algorithms and hyperspectral imaging has become a research hotspot for numerous scholars, as evidenced by references [19-21]. In this dynamic research field, multiple pioneering studies have emerged. For example, Ren et al. [22] ingeniously employed four deep learning models, incorporating coordinate attention mechanisms and label smoothing loss functions, to accurately detect soybean seed vigor. Yue et al. [23] developed LeafTraitNet based on the Visual Geometry Group architecture, integrating features from both deep and shallow networks to estimate the physicochemical parameters of plant leaves. Among various deep learning techniques, the Bidirectional Long Short-Term Memory Network (BiLSTM) is an enhanced version of the traditional LSTM. It has significant advantages in processing sequence data, as it can comprehensively extract deep temporal features from sequences, laying a crucial foundation for key extraction of complex spectral sequences in hyperspectral data [24]. Adaptive Boosting (Adaboost) is a well-established ensemble learning algorithm. It combines multiple weak learners into a strong learner, substantially improving the prediction accuracy and generalization ability of the model [25-27]. The BiLSTM - Adaboost collaborative model is a cutting-edge approach that integrates the dual advantages of bidirectional key extraction and ensemble optimization. It effectively breaks through the performance limitations of single models, enabling more accurate and reliable analysis. Currently, scholars have actively applied it in multiple fields [28-31]. However, there is still a significant research gap in the application of this collaborative model in the field of agricultural science, especially regarding the precise analysis of GABA and vitamin B9 content in foxtail millet, with no relevant research reported so far.

Materials and methods: Some paragraphs require citations to support the choice of methodology used. The methodology should be written directly, describing the steps in chronological order of how the procedures were performed and in a way that can be reproduced.

The scheme follows Syu, K. Y. (2008) [32] regarding the determination process of GABA using liquid chromatography.

In line 246-261: The Interval Random Frog (iRF) algorithm, based on the Random Frog partial least squares framework, updates variable subsets by leveraging the principles of survival of the fittest and random search. Through an iterative process, it ranks key bands by their selection probability, facilitates local information transfer, and is used to select optimal key bands [38]. The Interval Variable Iterative Space Shrinkage Algorithm (iVISSA) is a key selection method combining global and local search. By optimizing band weights via alternating search between global soft shrinkage and local continuous information, it determines the optimal position and width of variable intervals for modeling analysis [39]. The Competitive Adaptive Reweighted Sampling (CARS) algorithm employs adaptive reweighted sampling to emulate the "survival of the fittest" principle. Through multiple weighting rounds, it retains high-weight features, thereby screening strongly predictive variables from high-dimensional data [40]. The Bootstrapped Soft Shrinkage (BOSS) algorithm addresses inaccurate predictions caused by model collinearity. By statistically analyzing regression coefficients of sub-models and optimizing variable proportions through weighted bootstrapped sampling, it extracts the optimal variable set [41].

Results and discussion:

It is recommended to present the main differences observed in %. Depending on the modifications made to the results section, new citations can be added in the discussion to explain the results obtained.

In line 364-385: The wavelength range of 950–997 nm is primarily associated with the vibrational absorption of chemical bonds in components such as water and carbohydrates. This absorption represents the internal energy conversion within molecules, indicating their selective absorption characteristics for light of specific wavelengths [48]. The wavelength range of 997–1100 nm may correspond to reduced absorption of light by vibrational modes of certain groups, such as proteins or fats, within this interval. This demonstrates the modulatory effects of components like proteins and fats on the spectrum within this wavelength band [48]. The wavelength range of 1100–1199 nm likely reflects the enhanced combined absorption of carbohydrates like starch. Overlapping absorptions within this range strengthen light absorption, leading to decreased reflectance and highlighting the dominant absorption role of carbohydrates in this spectral region [43]. The wavelength range of 1199–1298 nm may be related to weakened absorption by certain functional groups, reflecting the influence of combination bands of functional group vibrations on spectral characteristics. The wavelength range of 1298–1468 nm is likely associated with water absorption. Significant absorption of light energy occurs here, causing a marked decrease in reflectance. This key underscores the critical influence of water on reflectance within this spectral interval. The wavelength range of 1468–1650 nm likely arises from the overlapping and interacting absorption characteristics of multiple components within millet grains, such as proteins, fats, and carbohydrates. Reflectance exhibits specific trends within this range, reflecting the complexity of millet's

composition and the composite nature of its spectral features [43][44].

In line 513-522: As shown in Figure 3, the wavelength range of 950–997 nm primarily corresponds to the transition between vibrational energy levels of O-H bonds in water molecules and C-H bonds in carbohydrates, where light absorption occurs. The 997–1100 nm range may be associated with vibrational modes of functional groups such as amide groups in proteins or C-H stretching vibrations in lipids. Within the 1100–1199 nm range, complex molecular chain structures contribute to vibrational absorption of bonds like O-H and C-O [43]. The 1199–1298 nm range likely relates to combination bands of C-H and O-H vibrations. In the 1298–1468 nm range, strong overtone absorption of O-H vibrations dominates. The 1468–1650 nm range may involve coupled stretching vibrations from overtones and combination bands of C-H, C-O, and O-H bonds.

In line 554-622: Using iRF-BOSS to select wavelengths highly concentrated in the strong absorption region of hydrogen-containing groups: 14 wavelengths (accounting for 70%) are distributed in the range of 1021 to 1124 nanometers, representing the most typical spectral characteristics of GABA molecules. These wavelengths exhibit intense signals and high specificity, directly related to the content of amino groups and methylene groups. Moreover, 6 wavelengths covering 1275–1327 nm retain core sensitive points for combination vibrations. They encompass peak positions in the combination band region (1299 nm, 1313 nm) [47], which are sensitive to the conformational changes of GABA molecules, thereby further enhancing the accuracy of concentration prediction. The refined wavelengths exist as discrete single points, all located at the vertices or sudden slope changes of the spectral absorption peaks. These serve as “informational anchor points” that maximally reflect the changes in GABA concentration, effectively avoiding the interference from redundant wavelengths in continuous intervals.

Using iVISSA-BOSS precisely focuses on specific spectral regions of the Vitamin B9 molecular core structure: 15 wavelengths (accounting for 60%) are distributed in the range of 959 - 1157 nanometers, mainly corresponding to the first overtones of C - H and N - H bonds. This represents the typical spectral key range for the aromatic and heterocyclic structures in folic acid molecules, with signals exhibiting significant specificity that directly correlates with the content information of key structures in folic acid. Within the core sensitive regions reflecting intramolecular interactions, 8 wavelengths are distributed across the 1318-1379 nm and 1398-1464 nm intervals. Among them, 1318-1379 nm is related to the second overtone vibration of the O-H bonds in the hydroxyl groups, which can sensitively capture the impact of the hydroxyl functional groups and water in the folic acid molecule on the spectral signals. The 1398-1464 nm range corresponds to the characteristic region of hydrogen bond interactions within the molecule, presenting a highly sensitive response to structural changes in the folic acid molecule. These wavelengths cover the key sites of intramolecular interactions, providing spectral support at the structural level for the precise prediction of Vitamin B9 concentration. Additionally, 2 wavelengths located at 1605-1648 nm are presumed to be related to the characteristic absorption of the glutamic acid moiety in the folic acid side chain, effectively supplementing the spectral representation of the overall structure of the molecule. Notably, all refined wavelengths exist as discrete single points, precisely located at the vertices or critical positions of

sudden slope changes in the spectral absorption peaks. This strategy avoids the interference from redundant wavelengths in continuous intervals, enabling the model to efficiently extract core spectral information relevant to concentration, thereby achieving accurate detection of Vitamin B9 content in foxtail millet. This wavelength selection strategy, focused on key informational anchor points, not only significantly enhances the model's prediction accuracy but also strengthens the reliability of the detection method in terms of stability and generalization capability.

The stacked BiLSTM-Adaboost model demonstrates significant advantages in GABA detection. Its core strength lies in fully leveraging BiLSTM's deep processing capability for spectral sequence information, enabling it to accurately capture latent dependencies between different wavelengths. Simultaneously, by utilizing Adaboost's ensemble learning mechanism, the model substantially enhances its fitting capacity and generalization ability for complex data. Vergara-Diaz O established a detection model for wheat NIR spectral data and GABA using PLS, achieving an $R^2 = 0.71$. Consequently, compared to traditional machine learning models, this stacked model can more efficiently handle the non-linear relationships and long-range dependencies within spectral data, significantly improving detection accuracy. In the Vitamin B9 detection, this model effectively captures the complex spectral features of folic acid molecules, markedly boosting prediction accuracy and stability. Compared to GABA detection, the key wavelengths for Vitamin B9 are more broadly distributed, involving more molecular structural characteristics, which places higher demands on the model's learning capability. The stacked BiLSTM-Adaboost model, with its unique architecture and ensemble learning advantages, successfully meets this requirement.

However, this study still has certain limitations. Firstly, the limited sample size—covering only 190 samples from 19 varieties across one production region—may affect the model's generalizability. Future research should expand the scope and quantity of samples to enhance the model's robustness. Secondly, potential issues in broader application exist: as foxtail millet from different regions varies in growth environments (e.g., soil, climate), its GABA and Vitamin B9 content and spectral characteristics may differ. The model's applicability to millet from other production areas requires further validation. Finally, limitations arise in real-time or field applications. The current detection process requires professionals to operate spectrometers and perform data preprocessing, making it challenging to meet the demand for rapid field detection. Subsequent efforts should integrate portable spectral devices and lightweight model algorithms to optimize the detection process and reduce detection time, thereby improving practicality.

Conclusion: It is recommended to write in a way that does not repeat the presentation of the results obtained. Write the conclusions obtained based on the results obtained.

This study focuses on the application of near-infrared spectroscopy (NIRS) technology for the non-destructive detection of γ -aminobutyric acid (GABA) and Vitamin B9 in millet. Innovatively, it proposes four key variable extraction strategies — iRF-CARS, iRF-BOSS, iVISSA-CARS, and iVISSA-BOSS — and establishes detection models centered on these strategies.

Initially, the study systematically analyzed the GABA and Vitamin B9 content across diverse millet varieties. The results indicated significant inter-varietal differences, which may be closely associated with factors such as the millet's genetic characteristics and environmental adaptability. These findings provide a critical foundation for the precise screening of nutrient-rich millet varieties.

Furthermore, the study integrates the four proposed key extraction strategies with a stacked BiLSTM-Adaboost model. The experimental results demonstrate that the key wavelength screening method introduced in this work effectively reduces data dimensionality and enhances critical information. Consequently, the stacked BiLSTM-Adaboost model exhibits significantly improved performance in detecting GABA and Vitamin B9 content in millet, achieving not only substantially higher prediction accuracy but also markedly enhanced model stability.

Future research will proceed in several directions:

- Expanding the sample size to encompass more millet varieties will facilitate investigation into the specific mechanisms by which genetic and environmental factors influence GABA and Vitamin B9 content, aiming to develop more robust variety screening models.
- For the stacked BiLSTM-Adaboost model, we plan to optimize its architecture and hyperparameters while exploring integration with other deep learning techniques to enhance its generalization capability and detection efficiency.
- Extending the research scope to include spectral detection of other nutritional components in millet, we will leverage existing key extraction strategies and modeling expertise to establish multi-component simultaneous detection models.

This integrated approach will provide comprehensive technical support for millet quality evaluation and functional food development.