# การประเมินปริมาณกรดแกมมา-อะมิโนบิวทิริกเบื้องต้นในผลิตภัณฑ์ข้าวด้วยวิธีสเปกโทรโฟโตเมตรีช่วงยูวี–วิสิเบิลแบบง่ายโดยใช้การทำอนุพันธ์ด้วย 2-Hydroxynaphthaldehyde

# A Simple UV–Vis Screening Method for Estimating GABA in Rice Products Using 2-Hydroxynaphthaldehyde Derivatization

**นิพนธ์ต้นฉบับ** **Original Article**


ตะวันฉาย โพธิ์หอม[1], บัญชา ยิ่งงาม[2] และ จารุวรรณ ธนวิรุฬห์[2]*

[1] ภาควิชาวิศวกรรมอุตสาหการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี อ.วารินชำราบ จ.อุบลราชธานี 34190
[2] กลุ่มวิชาเภสัชเคมีและเทคโนโลยีเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี อ.วารินชำราบ จ.อุบลราชธานี 34190

* Corresponding author: charuwan.t@ubu.ac.th



*วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ 2569;21(2):95-103.*



Tawanchai Phohom[1], Bancha Yingngam[2] and Charuwan Thanawiroon[2]*

[1] Department of Industrial Engineering, Faculty of Engineering, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand
[2] Department of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand

* Corresponding author: charuwan.t@ubu.ac.th



*Thai Pharmaceutical and Health Science Journal 2026;21(2):95-103.*



## บทคัดย่อ

**วัตถุประสงค์:** เพื่อพัฒนาและประเมินเบื้องต้นวิธีคัดกรองด้วยสเปกโทรโฟโตเมตรีช่วงยูวี–วิสิเบิลที่ง่าย รวดเร็ว และประหยัด สำหรับการประมาณปฏิกิริยาที่เกี่ยวข้องกับกรดแกมมา-อะมิโนบิวทิริก (GABA) ในผลิตภัณฑ์ข้าว โดยใช้การทำอนุพันธ์ด้วย 2-hydroxynaphthaldehyde (HN) **วิธีการศึกษา:** ตัวอย่างข้าว ได้แก่ ข้าวกล้อง ข้าวผสม ข้าวดำ และข้าวกล้องงอก (germinated brown rice; GBR) นำมาสกัดด้วยเอทานอล 75% จากนั้นทำปฏิกิริยากับ HN ปริมาตร 3 มิลลิลิตร ในสารละลายบัฟเฟอร์โบเรต (pH 8.0) ให้ความร้อนที่ 80 °C เป็นเวลา 15 นาที และวิเคราะห์ที่ความยาวคลื่น 415 นาโนเมตร **ผลการศึกษา:** สารอนุพันธ์ GABA–HN ให้แถบการดูดกลืนแสงที่ 415 นาโนเมตร ซึ่งสามารถใช้เป็นสัญญาณสำหรับการคัดกรองเบื้องต้นได้ กราฟมาตรฐานมีความเป็นเส้นตรงในช่วงความเข้มข้น 0.1 – 2.0 mg/mL ($y = 0.4543x + 0.1058$, $R^2 = 0.9967$) และมีความเที่ยงซ้ำที่ดี (RSD < 5%) แต่เนื่องจาก HN สามารถทำปฏิกิริยากับหมู่อะมิโนปฐมภูมิของสารอื่นได้ วิธีนี้จึงควรตีความเป็นการประเมินค่า apparent GABA–HN-equivalent response มากกว่าวิธียืนยันที่จำเพาะต่อ GABA ผลการประยุกต์ใช้พบว่า GBR มีค่า apparent GABA–HN-equivalent สูงที่สุด (0.7017 ± 0.0299 mg/g น้ำหนักแห้ง) ตามด้วยข้าวกล้อง (0.6902 ± 0.0109 mg/g น้ำหนักแห้ง) ความต่างนี้มีนัยสำคัญทางสถิติ (P-value < 0.05) แต่มีขนาดผลที่ค่อนข้างน้อย **สรุป:** สามารถใช้วิธี HN–UV เป็นเครื่องมือคัดกรองเบื้องต้นในผลิตภัณฑ์ข้าว แต่เนื่องจาก HN สามารถทำปฏิกิริยากับสารที่มีหมู่อะมิโนปฐมภูมิได้ จึงควรตีความผลเป็นค่าประมาณหรือค่าเทียบเท่า GABA–HN ภายใต้เงื่อนไขการทดลอง และต้องตรวจสอบความจำเพาะ การคืนกลับ ผลของเมทริกซ์ ความคงทนของวิธี และการเปรียบเทียบกับวิธีอ้างอิงทางโครมาโทกราฟีเพิ่มเติมก่อนใช้เป็นวิธีวิเคราะห์เชิงยืนยัน



**คำสำคัญ:** กรดแกมมา-อะมิโนบิวทิริก; ยูวี–สเปกโตรโฟโตเมทรี; 2-ไฮดรอกซีแนฟทัลดีไฮด์; ข้าวกล้อง; ข้าวผสม; ข้าวดำ; ข้าวกล้องงอก

Editorial note
*Manuscript received in original form: April 21, 2026;*
*Revision notified: June 15, 2026;*
*Revision completed: June 19, 2026;*
*Accepted in final form: June 29, 2026;*
*Published online: June 30, 2026.*



## Abstract

**Objective:** To develop and preliminarily evaluate a simple, rapid, and economical UV–visible spectrophotometric screening method for estimating γ-aminobutyric acid (GABA)-associated reactions in rice products using 2-hydroxynaphthaldehyde (HN) derivatization. **Method:** Rice samples (brown, mixed, black, and germinated brown) were extracted with 75% ethanol, derivatized with 3 mL of HN in borate buffer (pH 8.0), heated at 80 °C for 15 min, and analyzed at 415 nm. **Results:** The GABA–HN derivative produced a visible absorption band at 415 nm, which was selected as the analytical wavelength for preliminary screening. The calibration was linear from 0.1 – 2.0 mg/mL ($y = 0.4543x + 0.1058$, $R^2 = 0.9967$), with good precision (RSD < 5%). When applied to real samples, germinated brown rice had the greatest apparent response (0.7017 ± 0.0299 mg/g DW), although the difference from brown rice was small (0.6902 ± 0.0109 mg/g DW) and remained statistically significant (P-value < 0.05). **Conclusion:** The UV–visible spectrophotometric screening approach may be useful for preliminary quality screening of functional rice products in laboratories with limited access to chromatography. However, comprehensive selectivity testing against rice amino acids, matrix-matched recovery or standard-addition experiments, ruggedness testing, and cross-validation against HPLC are needed before the method can be used for confirmatory quantification.



**Keywords:** gamma-aminobutyric acid; UV spectrophotometry; 2-hydroxynaphthaldehyde; brown rice; mixed rice; black rice; germinated brown rice

Journal website:
http://ejournals.swu.ac.th/index.php/pharm/index


## Introduction

Gamma-aminobutyric acid (GABA) is a nonprotein amino acid and the main inhibitory neurotransmitter in the mammalian central nervous system. The reported health benefits of GABA, including stress reduction, sleep quality improvement, and antihypertensive, antidiabetic and anti-inflammatory effects, have increased interest in functional food applications.[1-4] Recent studies have also shown its potential neuroprotective, anxiolytic and gut–brain axis modulatory effects.[1-3]

Rice, especially brown and pigmented rice, is still the main dietary source of GABA. During the germination process, the activity of glutamate decarboxylase is initiated, resulting in the

conversion of glutamate to GABA and rapid biochemical build-up of this amino acid within the grain.[5-8] On the other hand, compared with ungerminated seeds, germinated brown rice (GBR) has significantly higher concentrations of GABA, normally in the range of 20–40 mg/100 g dry weight (DW), under standard conditions for GBR preparation.[6,9] By altering parameters through well-controlled soaking, specific temperature and duration shifts or careful stress-induction treatments, the values of these parameters can all be elevated beyond 80 – 100 mg/100 g DW.[6] As a result, already high baseline levels of GABA biosynthesis are observed in many pigmented cultivars—exemplified by black and purple rice—which contain higher concentrations of secondary metabolites (phenolics or anthocyanins) than their nonpigmented counterparts do. These compounds can also function as precursors for the generation of optimum levels or even increase advancement during germination to facilitate redox status homeostasis.[8,10] Exploiting this unique biochemical matrix, commercial products have recently been launched in the retail market and are often marketed as “GABA rice” and are more broadly viewed as increasingly attractive functional foods.[11] Such market expansion necessitates the establishment of reliable and validated analytical methods during industrial processing, as well as the quantification of actual GABA contents through labeling.[12,13]

GABA quantification by high-performance liquid chromatography (HPLC) and gas chromatography (GC) is routinely performed. However, these methods often require cumbersome precolumn derivatization or specialized detector configurations.[5,14] Despite these procedural limitations, HPLC with fluorescence or UV detection after derivatization continues to be the most sensitive reference standard.[5,14] With respect to the most common amino acid analytical derivatizing agents, o-phthalaldehyde (OPA), ninhydrin and naphthalene-based aldehydes such as OPA display good synthetic analytical sensitivity, but the derived isoindole species are too unstable to allow for delayed analyses.[15] Ninhydrin is also a conventional reagent used to identify amino acids, but its chromogenic reaction is not strictly substrate specific and can therefore be interfered with by endogenous compounds common in complex food matrices.[16] In contrast, 2-hydroxynaphthaldehyde (HN) can generate more stable Schiff base adducts with primary amino groups with significant visible absorption, therefore allowing simpler analytical processing. Chromatographic procedures possess analytical advantages but are generally time-consuming and expensive and rely on sophisticated apparatuses that may not be easily accessible in ordinary quality-controlled laboratories. Hence, there is a growing demand for simpler, faster and more cost-effective solutions for the determination of GABA in functional food products.

UV–visible spectrophotometry is typically used in food and pharmaceutical facilities. However, direct detection of GABA is limited since the molecule lacks a native chromophore. Therefore, derivatization is required to generate a visible signal. A possible technique is derivatization with HN. The reagent reacts with the primary amino groups of the compounds to form Schiff base adducts that can also be quantified in the visible spectrum.[9] On the basis of this reaction mechanism, Hayat et al.[17] and Sharma[9] quantified GABA after HN derivatization. Nonetheless, the link to practical application is still rather narrow. Thus, further analytical validation is needed to accommodate various rice cultivars, representative germination conditions and more challenging sample matrices.

The aim of this work was to establish and assess a quick UV–visible spectrophotometric screening approach based on HN derivatization for the indirect estimation of the GABA-related response in rice matrices. This cost-effective approach represents a useful screening method complementary to conventional chromatographic methods, which are often reliant on expensive instrumentation, unique reagents, and highly skilled bench practitioners. The increasing use of GABA-rich rice, particularly GBR, as an ingredient for functional foods warrants the availability of relevant simple quality control methods across multiple product categories. This study provides meaningful analytical insights by developing a standard sample preparation approach for amino acid analyses from different rice matrices under conventional benchtop conditions. However, it should be noted that as HN is a nonselective reagent that reacts with primary amino groups other than GABA alone, the proposed assay can play only an advisory semiquantitative screening role but not a confirmatory analytical role. In conclusion, our results revealed the value of simple spectrophotometric methods for the preliminary evaluation of GABA-enriched rice, taking into account practicality in operation against inherent analytical limitations.

## Methods

***Chemicals and reagents***

γ-Aminobutyric acid (GABA, purity ≥ 99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). 2-Hydroxynaphthaldehyde (HN, purity ≥ 98%) was obtained from Merck (Darmstadt, Germany). Ethanol (analytical grade, ≥ 99.5%) and all other reagents were purchased from Carlo Erba Reagents (Milan, Italy). Deionized water was prepared via a Milli–Q purification system (Millipore, Burlington, MA, USA).

A 0.1 M borax buffer (sodium tetraborate, pH 8.0) was freshly prepared and used to provide alkaline conditions for derivatization. A GABA stock solution (10 mg/mL) was prepared in Milli-Q water and stored at 4 °C. Working standard solutions (0.1 – 2.0 mg/mL) were freshly prepared by serial dilution from this stock solution. The HN derivatization reagent was prepared as a 0.3% (w/v) ethanolic solution of HN and stored in amber glass bottles at 4 °C to prevent light-induced degradation.

***Sample preparation***

In this study, four kinds of rice samples were analyzed: brown rice, mixed rice, black rice and germinated brown rice. Brown rice and black rice (whole grain and bran) were obtained from a local cooperative in Ubon Ratchathani Province, Thailand. The mixed rice sample was prepared by combining brown rice and black rice at a 1:1 ratio by weight (w/w). The two rice samples were weighed in equal proportions, mixed thoroughly, and then processed under the same grinding and extraction conditions as the other rice samples. Brown rice was germinated in-house by soaking brown rice in distilled water at a 1:5 (w/v) ratio at room temperature (28 ± 2 °C) for 24 – 48 h under humid circumstances until visible sprouts developed. The germinated grains were subsequently dried in a hot-air oven at 50 °C (Memmert, Schwabach, Germany).

Dried rice samples were finely ground in a Cyclotec Sample Mill (FOSS, Hillerød, Denmark) with a 0.5 mm mesh. Each powdered sample (2.00 ± 0.01 g) was extracted with 20 mL of 75% ethanol (v/v) in a 50 mL conical flask using an orbital shaker (IKA KS 4000, Staufen, Germany) at 150 rpm for 1 h at room temperature. Although GABA is highly soluble in water, a hydroalcoholic extraction solvent of 75% ethanol–water was chosen because the aqueous phase favors the extraction of polar, free GABA, whereas the ethanol phase reduces the coextraction of starch, proteins and other high-molecular weight compounds that cause turbidity or background absorbance in the UV–visible spectrum. After extraction, the mixture was centrifuged for 10 min at 5000 rpm using an Eppendorf 5804R centrifuge (Hamburg, Germany). The supernatant was filtered using Whatman No. 1 filter paper (GE Healthcare, Chicago, IL, USA). The residue was re-extracted again under the same conditions, and the resulting filtrates were pooled. The pooled extracts were kept at 4 °C until analysis. This re-extraction process was implemented to increase extraction efficiency and reduce variability, which is especially crucial for reproducibility in complex food matrices.

***Derivatization procedure***

Aliquots of each extract or standard solution (1.0 mL) were placed into a 10 mL glass test tube. Afterward, 1.0 mL of borate buffer (pH 8.0) and 3.0 mL of HN reagent were added to bring the volume to 5.0 mL. The mixture was then vortexed for 30 s using a Vortex-Genie 2 mixer (Scientific Industries, Bohemia, NY, USA) and heated in a thermostatically controlled water bath (Julabo, Seelbach, Germany) at 80 °C for 15 min. A reagent blank with borate buffer and HN reagent in the absence of GABA was produced and processed in parallel. The proposed reaction involves condensation of the aldehyde group of HN with the primary amino group of GABA under mild alkaline conditions to yield a visible-absorbing imine or Schiff base derivative (Figure 1).

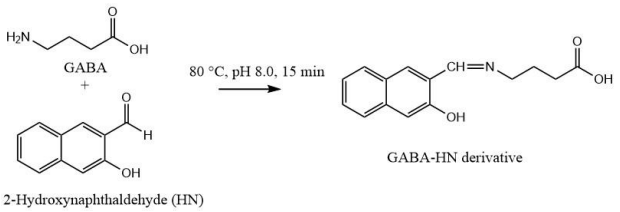


**Figure 1** Proposed HN–GABA reaction to synthesize a GABA–HN Schiff base derivative. This structure is suggested to explain color production and does not by itself provide analytical specificity for GABA in rice matrices.

The optimized derivatization parameters, i.e., a pH of 8.0, 3.0 mL of HN and heating at 80 °C for 15 min, were determined on the basis of preliminary single-factor studies, in which the appropriate chromophore signal intensity was determined under the tested conditions. After heating, the

reaction mixtures were cooled to room temperature, and the absorbance was measured within 30 min in the same analytical run. A limitation of the present study is that the extended postreaction color stability across defined time periods was not completely assessed.

***Spectrophotometric measurements***

The absorbance spectra were measured in the 250–600 nm range using a UV–visible spectrophotometer (Shimadzu UV-2101PC, Kyoto, Japan) and 1 cm quartz cuvettes (Hellma Analytics, Müllheim, Germany). The visible absorption band of the GABA-HN derivative was at 415 nm and was chosen for analytical analysis. In the 310–370 nm band, the absorbance was higher, but the lower wavelength region had more overlap with the HN-related absorbance and probable background signals from the rice matrix. On the other hand, compared with the reagent blank, the peak at 415 nm resulted in a better reaction in the visible region and was more suitable for regular screening of derivatized rice extracts. Under the investigated conditions, the underivatized extracts had little absorbance at 415 nm.

***Method validation***

Analytical performance was evaluated preliminarily against validation features as defined in ICH Q2(R2), paying attention to linearity and repeatability as required for a screening assay.[18] Linearity was established over a concentration range of 0.1–2.0 mg/mL using least-squares regression. The precision was determined as the relative standard deviation (RSD %) from replicate examinations of both the standard solutions and the rice extracts. Several validation aspects could not be fulfilled in this preliminary evaluation. These included full determination of the limit of detection and quantification (LOD/LOQ), specificity testing for other amino acids, spike-recovery or standard-addition studies for assessment of accuracy, and matrix-matched calibration and ruggedness testing on different instruments or analysts. These omissions are therefore accepted as limitations and should be addressed in future validation investigations.

***Statistical analysis***

All analyses were performed using three independent sample preparations. Each extract was measured in triplicate, and the technical triplicate readings were averaged before statistical analysis. Data are expressed as the mean ± standard deviation (SD) of the independent preparations. Differences among apparent GABA–HN-equivalent values were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test when appropriate. Statistical significance was defined as $p < 0.05$. Because the assay was preliminarily evaluated and the number of independent preparations was limited, statistical comparisons should be interpreted as exploratory.

## Results

***Optimization of Derivatization Conditions***

The derivatization conditions were adjusted preliminarily to obtain a clear visual absorbance signal for the GABA-HN derivative. The effects of three reaction parameters (volume of the HN reagent, reaction pH and heating time/temperature) were studied. These factors were selected because the creation of a Schiff base is influenced by the availability of reagents, alkaline reaction conditions and heat-assisted condensation. The highest practicable absorbance response at 415 nm was obtained using 3 mL of HN, while lower reagent volumes (1 – 2 mL) resulted in weaker color development (Figure 2).

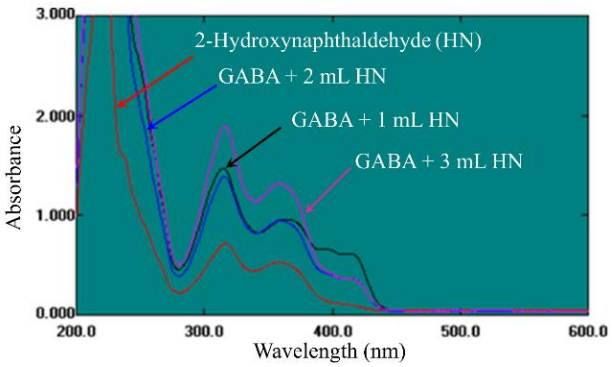


**Figure 2** UV–visible absorption spectra of the GABA–HN derivative and reagent blank. The spectra of a derivatized GABA standard (50 µg/mL after reaction and dilution) exhibit a strong absorption peak at 415 nm (green, black and violet lines), whereas the reagent blank (HN + buffer without GABA; red line) has little absorbance at this wavelength. This result justified the use of 415 nm as a realistic wavelength in the visible region for preliminary screening, despite the analytical specificity not being complete.

No obvious increase was observed when the volume of the HN reagent was greater than 3 mL under the tested conditions. The influence of pH was subsequently examined in the range of 7.0 – 10.0 using borate buffer, as shown in

Figure 3. The maximum absorbance occurred at a pH of 8.0, and lower responses were observed at higher pH values. Additionally, the temperature conditions were studied, and incubation at 80 °C for 15 min resulted in a uniform response. Shorter heating times (5 – 10 min) resulted in lower absorbance, indicating incomplete color development. Considering these preliminary experiments, the following derivatization conditions were chosen: 3 mL of HN, pH 8.0 and heating at 80 °C for 15 min. These conditions were subsequently used for the analysis. Notably, these are preliminary single-factor condition selections and not a full multivariable optimization of the analytical procedure.

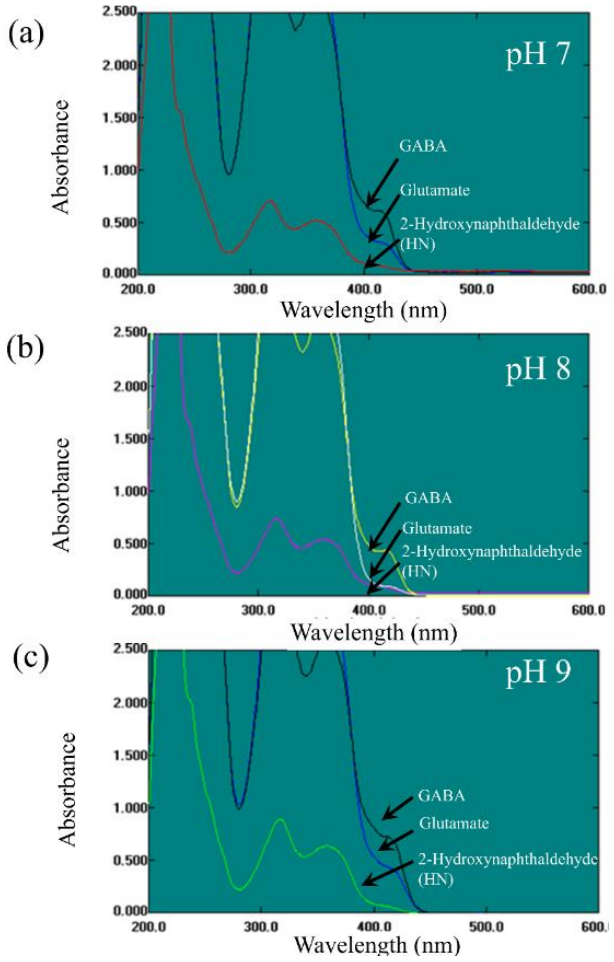


**Figure 3** Effect of solution pH on the derivative absorbance of the GABA-HN reaction. The highest reaction was obtained in the pH range of 8–9, and a pH of 8 was selected for the borate buffer technique. Each point represents the mean of three derivatizations using a 50 µg/mL GABA standard. The color development increased with time and plateaued at approximately 15 min, which was selected as the reaction time.

### *Absorption Spectrum and Wavelength Selection*

The UV–vis absorption spectrum of the GABA-HN derivative obtained under the above reaction conditions showed a distinct absorption band at 415 nm. In the 310–370 nm range, peaks or shoulders of higher strength were observed, although this band is more susceptible to influence by the absorbance of the reagent and the natural background signals from the rice matrix. However, the absorbance of the reagent blank was relatively low at 415 nm, which is more suitable for regular screening. Therefore, the analytical wavelength was set at 415 nm. This option enhances the practical utility of the test but should not be considered to prove 100% specificity for GABA. Other important amino compounds may similarly react with HN to yield absorbent derivatives.

### *Calibration curve and linearity*

The calibration curves constructed with the GABA standards in the concentration range of 0.1–2.0 mg/mL exhibited satisfactory linearity. The regression equation was y = 0.4543x + 0.1058, with an $R^2$ value of 0.9967, where y is the absorbance at 415 nm and x is the GABA concentration in mg/mL. The strong correlation coefficient implies a proportional response of standard solutions in the measured range. However, the analytical performance should be considered preliminary because the LOD, LOQ, matrix-matched recovery and cross-reactivity tests were not performed. Therefore, the method is deemed to be applicable for screening purposes rather than for confirming quantification. The preliminary validation findings and remaining validation items are summarized in Table 1.

**Table 1** Summary of the preliminary analytical performance and validation limitations of the HN–UV method.

| Validation characteristic | Results in this study | Interpretation/limitation |
|---|---|---|
| Linearity | 0.1 – 2.0 mg/mL; y = 0.4543x + 0.1058; $R^2$ = 0.9967 | Acceptable proportionality within the tested standard range. |
| Repeatability/precision | Rice sample RSD values: 1.58 – 4.26% | Acceptable for preliminary screening. |
| LOD | Not established in the present dataset | Requires blank/noise or low-level replicate experiments. |
| LOQ | Not established in the present dataset | Required before claiming quantitative sensitivity. |
| Accuracy/recovery | Not evaluated by spiking or standard addition | Needed to prove reliability in rice matrices. |
| Selectivity | Partially assessed by reagent blank and native extract spectra only | Cross-reactivity against amino acids in rice remains to be tested. |
| Ruggedness/robustness | Not evaluated across instruments, analysts, or days beyond preliminary repeatability | Needed before confirmatory quality control use. |

### *Apparent GABA–HN-equivalent values in rice samples*

The developed method was applied to estimate apparent GABA–HN-equivalent values in the four rice samples. The

apparent values (on a dry weight basis) determined for brown rice, mixed rice, black rice, and germinated brown rice are summarized in Table 2. Brown rice contained 0.6902 ± 0.0109 mg/g (RSD 1.58%), the mixed rice product contained 0.5283 ± 0.0128 mg/g (RSD 2.42%), black rice contained 0.5062 ± 0.0112 mg/g (RSD 2.21%), and germinated brown rice contained 0.7017 ± 0.0299 mg/g (RSD 4.26%). Statistical analysis confirmed that compared with the other samples, the germinated brown rice sample had a significantly greater apparent GABA–HN-equivalent value ($p < 0.05$). Although the difference between germinated brown rice and brown rice was small (~0.01 mg/g DW), it was statistically significant under the present experimental conditions. The relative standard deviations for all the rice determinations were less than 5%. These results describe the observed sample response under the HN–UV–Vis conditions used in this study.

**Table 2** Apparent GABA–HN-equivalent values of the rice samples determined by the HN–UV–Vis screening method at 415 nm.

| Sample | Apparent GABA–HN-equivalent value (mg/g DW) | RSD (%) | Significance |
|---|---|---|---|
| Brown rice | 0.6902 ± 0.0109 | 1.58 | b |
| Mixed rice (brown rice:black rice, 1:1 w/w) | 0.5283 ± 0.0128 | 2.42 | c |
| Black rice | 0.5062 ± 0.0112 | 2.21 | c |
| Germinated brown rice | 0.7017 ± 0.0299 | 4.26 | a |

**Note:** Method conditions: extraction with 75% ethanol; derivatization with 0.3% (w/v) HN in borate buffer, pH 8; 80 °C for 15 min; measurement at 415 nm against the reagent blank.

The values are presented as the mean ± SD (mg/g DW). Each sample was prepared independently in triplicate, and each extract was measured in triplicate, resulting in a total of nine absorbance readings per sample. Different superscript letters within a column indicate significant differences according to one-way ANOVA followed by Tukey's post hoc test (P-value < 0.05).

## Discussions and Conclusion

This study demonstrated that a simple UV–spectrophotometric assay based on HN derivatization can be used as a preliminary screening approach to estimate apparent GABA–HN-equivalent responses in rice products with linearity over 0.1 – 2.0 mg/mL ($R^2 = 0.9967$) and good precision (RSD < 5%) when widely accessible instruments and reagents are used. Among the four product types, GBR had the greatest apparent response, followed by brown rice, mixed rice, and black rice under the present conditions. These findings are consistent with the biological plausibility that germination activates glutamate decarboxylase activity and redirects glutamate flux toward GABA.[6,19-21] However, because HN is not inherently specific to GABA, the values should be interpreted as apparent GABA–HN-equivalent responses rather than fully confirmed GABA concentrations.

Our findings align with recent reports that germination—or germination coupled with mild process intensification—consistently elevates GABA levels in rice. For example, controlled soaking/germination regimes in rice substantially increased the GABA content while influencing factors such as temperature, time, and seed conditions,[22] and pulsed-light–assisted germination further promoted sprouting efficiency and GABA enrichment through metabolic reprogramming, as evidenced by multiomics.[21] A 2025 study in which germination was extended into parboiling workflows similarly reported higher levels of GABA alongside broader nutritional gains.[19] Although germinated brown rice contains the greatest amount of GABA, the absolute increase in GABA in brown rice is limited. These findings indicate that while germination significantly increases GABA biosynthesis, the nutritional impact of this process may depend on the conditions and processing factors involved.[20,23-25]

Methodologically, the proposed HN–UV method is a practical alternative to chromatographic platforms in preliminary screening. HPLC procedures with pre- or postcolumn derivatization are still the reference techniques for confirmatory analysis but are more expensive and require specialized instrumentation and technical skills. In contrast, the HN-derived product detected at 415 nm exhibited a usable visible-region response with little sample pretreatment and a quick reaction time of 15 min at 80 °C. However, wavelength selection alone cannot prove perfect specificity because HN may also react with other amino-containing chemicals contained in the rice matrix. Recently, the literature has highlighted the growing demand for cost-effective analytical procedures to improve the processing and release of GABA-enriched meals.[22,26] Improvements in colorimetric and derivatization chemistry, such as improved ninhydrin-based methods for free amino acid analysis, demonstrate how routinely used spectrophotometric methods can be adapted for complex food matrices.[2,4,16,24]

The uniqueness of this work is not in the introduction of HN derivatization per se but in establishing its practical utility in multiple rice varieties under ordinary laboratory circumstances. Chromatographic methods are still the gold standard for confirmatory quantification, but the proposed HN–UV method is a cheap and reproducible alternative for initial

screening. This could be particularly valuable for nutraceutical quality control laboratories, small university laboratories and food enterprises with limited access to modern analytical instruments. This method can provide consistent results across different kinds of rice. Thus, this study provides a feasible foundation for routine screening and quality assurance of functional grain products.[21,24] However, the procedure still needs more validation to be considered a confirmatory analytical technique.

From an analytical point of view, three points are worth mentioning. The 415 nm wavelength was chosen initially because it gave a more discrete response in the visible area than did the reagent blank or possible matrix background, but higher absorbance values were obtained in other spectral regions. Second, the approach exhibited a linear response and acceptable analytical precision within the examined concentration range. Third, the pH and volume of HN were selected from preliminary single-factor studies. These settings are in line with the expected behavior of aromatic aldehyde–amine condensation processes and broadly comparable with practical optimization ranges described in recent process-development studies.[22] Overall, the results suggest that the approach may be beneficial as a screening tool. However, they do not yet achieve full selectivity, accuracy and resilience in different sample matrices.[6,20,25,27]

The order of the rice sample ranking is consequently of direct relevance for nutritional profiling and for the further development of unique goods. The robust analytical signals suggest that germinated brown rice may be a good candidate as a functional food ingredient, including for manufacturing uses where there are specific consumer preferences (e.g., GABA identity blends, specific nutrient profiles) or markets that require clear delineation between potential products (specialty mandates). In contrast, the lower signal for mixed rice varieties is most likely a simple dilution effect with the potential to include polished grain fractions in these samples. Recent work on value-added germinated brown rice further supports this prospect, with GBR-based products with increased GABA content described as promising commercial formats.[26] Moreover, Braga et al.[2] emphasized the greater implications of dietary GABA in the context of brain and immunological functioning, highlighting the necessity for reliable but accessible analytical control. Thus, the suggested HN–UV approach can be an effective tool for iterative product optimization, routine screening, and supporting actual front-of-pack claims for GABA-enriched rice products.[26,28]

There are a number of strengths and limitations of this study. The strength of the approach lies in its simplicity and its appropriateness for ordinary laboratory use. The assay showed good linearity and acceptable precision (RSD <5%), indicating that the assay was suitable for preliminary screening and routine decision-making. The independence of the extractions and triplicate measurements also improved the reliability of the accuracy estimations at the product level, but some limitations still remain. No extensive cross-reactivity testing was performed with common amino acids in the rice matrices, such as glutamate, alanine, glycine, glutamine, asparagine, lysine and arginine. The study did not include matrix-matched calibration or standard-addition recovery procedures to investigate matrix effects. Furthermore, the evaluation of color stability was limited to the defined measurement window, and the method was not compared to a chromatographic reference with respect to the same sample lots. These limitations do not diminish the primary conclusions of the present investigation, but they provide clear priority for additional evaluation. Addressing these issues in future work would improve the analytical robustness of the method and reach the level of validation.[16,24]

Future studies should take advantage of the low cost and availability of the existing approach and further increase the analytical reliability. Selectivity against amino-rich interferents often seen in cereal and legume matrices should be further characterized by reporting the percentage of the GABA response for cross-reactivity. Matrix effects can be evaluated by spike-recovery protocols, which can be performed either by matrix-matched calibration or by standard-addition methods. The detection and quantification limit should be determined from replicate analyses of procedural blanks or, where appropriate, low-concentration samples. The time stability of the developed color should be checked in a strict temporal dimension before measurement confirmation since the photometric response is based on chromogenic derivatization. The robustness of the assay should be tested under interday and interoperator conditions to evaluate the performance of the assay under routine laboratory conditions. Cross-validation with HPLC on matched rice samples is needed to confirm the accuracy of the method and to define its practical role as a preliminary screening tool. These steps increase the method from an effective preliminary QC screen to a more broadly

validated analytical tool. They align with contemporary process-optimization studies in the corpus [22] and with product-oriented translational priorities emphasized by other groups.[2,19,21]

In summary, using only the data generated here, HN-based UV spectrophotometry provides a rapid and economical preliminary screening approach for estimating apparent GABA-related responses in rice products and captures germination-associated differences in the GBR. However, owing to the semispecific nature of HN derivatization and the absence of matrix recovery and chromatographic confirmation, the method should not yet be regarded as a fully validated confirmatory technique for definitive GABA quantification.

In conclusion, in this study, a simple and affordable UV–spectrophotometric screening method using HN derivatization was developed and preliminarily evaluated for estimation of apparent GABA–HN-equivalent responses in rice products. The selected preliminary operating conditions—3 mL of HN, borate buffer at a pH of 8.0, and heating at 80 °C for 15 min—yielded a visible chromophore with a maximum absorbance at 415 nm. The method demonstrated strong linearity in the concentration range of 0.1–2.0 mg/mL ($R^2$ = 0.9967) and good precision, with RSD values of less than 5%. These results suggest that it can be used as an initial screening tool. Compared with that of brown rice, the apparent response of germinated brown rice was the greatest among the studied samples, but the difference was quite moderate. However, these results should be considered with caution. The lack of specificity of HN derivatization and a comprehensive assessment of selectivity, matrix recovery, ruggedness, LOD/LOQ and chromatographic confirmation were not performed. This method should therefore be considered a preliminary semiquantitative screening assay rather than a fully validated confirmatory method. Therefore, future research should incorporate additional validation, including cross-reactivity, spike recovery, ruggedness, color stability and direct comparison with recognized chromatographic techniques.

**Acknowledgment**

The authors gratefully acknowledge the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand, for providing laboratory facilities and the technical support essential to this research.

**Author contributions**

**T.P.**: Investigation, Resources, Data curation, Validation, Visualization; **B.Y.**: Data curation, Writing – Original Draft; **C.T.**: Conceptualization, Methodology, Investigation, Data curation, Validation, Formal analysis, Supervision, Writing – Review & Editing, Project administration.

# References

1. Almutairi S, Sivadas A, Kwakowsky A. The effect of oral GABA on the nervous system: potential for therapeutic intervention. Nutraceuticals 2024; 4(2):241-259. (doi: 10.3390/nutraceuticals4020015)
2. Braga JD, Thongngam M, Kumrungsee T. Gamma-aminobutyric acid as a potential postbiotic mediator in the gut–brain axis. NPJ Sci Food 2024; 8(1):16. (doi: 10.1038/s41538-024-00253-2)
3. Hou D, Tang J, Feng Q, et al. Gamma-aminobutyric acid (GABA): a comprehensive review of dietary sources, enrichment technologies, processing effects, health benefits, and its applications. Crit Rev Food Sci Nutr 2024;64(24):8852-8874. (doi: 10.1080/10408398.2023.2204373)
4. Icer MA, Sarikaya B, Kocyigit E, et al. Contributions of gamma-aminobutyric acid (GABA) produced by lactic acid bacteria on food quality and human health: current applications and future prospects. Foods 2024;13(15):2437. (doi: 10.3390/foods13152437)
5. Chamsai T, Wanyo P. Enhancing GABA and antioxidant bioaccessibility in germinated brown rice: ultrasonic-assisted cellulase pretreatment optimized via RSM and ANN-GA. ACS Omega 2025;10(29):32080-32096. (doi: 10.1021/acsomega.5c03787)
6. Tsou S-F, Hsu H-Y, Chen S-D. Effects of different pretreatments on the GABA content of germinated brown rice. Appl Sci 2024;14(13):5771. (doi: 10.3390/app14135771)
7. Xu L, Wang X, Li Q, et al. Optimization of γ-aminobutyric acid production in brown rice via prolonged seed priming. Plants 2024;13(24):3594. (doi: 10.3390/plants13243594)
8. Yamuangmorn S, Saenjum C, Prom-u-thai C. Germination alters the bioactive compounds of pigmented and nonpigmented rice varieties in fresh and year-old stored seeds. Food Chem X 2024;24:102005. (doi: 10.1016/j.fochx.2024.102005)
9. Sharma R. Determination of gamma aminobutyric acid: ultraviolet spectroscopic method with 2-hydroxynaphthaldehyde derivatization. J Adv Nanotechnol Its Appl 2024;6(2):7-13. (doi: 10.5281/zenodo.11203126)
10. Yu Y, Li M, Li C, et al. Accelerated accumulation of γ-aminobutyric acid and modifications on its metabolic pathways in black rice grains by germination under cold stress. Foods 2023;12(6):1290. (doi: 10.3390/foods12061290)
11. Jabeen R, Jan N, Naseer B, et al. Development of germinated-brown-rice-based novel functional beverage enriched with γ-aminobutyric acid: nutritional and biofunctional characterization. Foods 2024;13(8):1282. (doi: 10.3390/foods13081282)
12. Bai Q, Liu S, Xu J, Tufail T, Xu B. Enrichment of GABA content in brown rice through heating and humidifying treatment: quantification via TLC-ImageJ method. Food Chem 2025;473:143033. (doi: 10.1016/j.foodchem.2025.143033)
13. Lim M-J, Barathikannan K, Jeong Y-J, et al. Exploring the impact of fermentation on brown rice: health benefits and value-added foods—a

comprehensive meta-analysis. Fermentation 2024;10(1):3. (doi: 10.3390/fermentation10010003)

14. Wang C-F, Huang C-R, Lu Y-C. Changes of antioxidant and functional components in various salt-aged and fresh radishes after fermentation. Fermentation 2024;10(9):479. (doi: 10.3390/fermentation10090479)
15. Xiao C, Xiao J, Wu Y, et al. Determination of amino acids of novel food in food by HPLC coupled with precolumn derivatization. Foods 2024; 13(24):4012. (doi: 10.3390/foods13244012)
16. Stauß AC, Fuchs C, Jansen P, et al. The ninhydrin reaction revisited: optimization and application for quantification of free amino acids. Molecules 2024;29(14):3262. (doi: 10.3390/molecules29143262)
17. Hayat A, Jahangir TM, Khuhawar MY, Alamgir M, Siddiqui AJ, Musharraf SG. Simultaneous HPLC determination of gamma amino butyric acid (GABA) and lysine in selected Pakistani rice varieties by precolumn derivatization with 2-hydroxynaphthaldehyde. J Cereal Sci 2014;60(2):356-360. (doi: 10.1016/j.jcs.2014.05.011)
18. International Council for Harmonization (ICH). ICH Q2(R2) guideline on validation of analytical procedures. Step 5. 2023. (Accessed on Jun. 15, 2024, at https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf)
19. Febina M, Deepa J, Maya R, et al. Combined effects of germination and parboiling on the nutrient bioavailability and glycemic index of selected indica rice varieties. Sci Rep 2025;15(1):23342. (doi: 10.1038/s41598-025-97165-2)
20. Saikia J, Borah P, Borah D, et al. Effects of germination on γ-aminobutyric acid (GABA) content and cooking quality of ahu rice landrace. J Food Sci 2025;90(8):e70501. (doi: 10.1111/1750-3841.70501)
21. Zhang G, Xu J, Wang Y, et al. A multiomics study revealed the effect of pulsed light treatment on germinated brown rice: promotion of sprouting efficiency and gamma-aminobutyric acid enrichment. Food Biosci 2024; 61:104196. (doi: 10.1016/j.fbio.2024.104196)
22. Lu W-C, Cheng Y-T, Chan Y-J, Yan J, Li P-H. Effects of different soaking and germinating conditions on γ-aminobutyric acid, antioxidant activity, and chemical composition of djulis (Chenopodium formosanum). J Agricult Food Res 2024;17:101162. (doi: 10.1016/j.jafr.2024.101162)
23. Sonsomboonsuk S, Junyusen T, Moolkaew P, et al. Effects of cooking methods on the physicochemical, textural and microstructural properties of hot salt puffed germinated brown rice. J Agricult Food Res 2024;15: 101001. (doi: 10.1016/j.jafr.2024.101001)
24. Tufail T, Ain HBU, Virk MS, et al. GABA (γ-aminobutyric acid) enrichment and detection methods in cereals: unlocking sustainable health benefits. Food Chem 2025;464:141750. (doi: 10.1016/j.foodchem.2024.141750)
25. Ukpong ES, Okpalanma EF, Ezegbe CC. Effect of milling and temperature of germination on nutrients, bioactive compounds and pasting properties of FARO 44, FARO 57 and NERICA-8 brown rice cultivars. Food Chem Adv 2024;4:100616. (doi: 10.1016/j.focha.2024.100616)
26. Tram HTN, Van Thinh P, Minh TN, et al. Enhanced production of gamma-aminobutyric acid (GABA) in Mang Buk Brown rice via optimal fermentation conditions with *Lactobacillus brevis*, *Lactobacillus pentosus*, and *Lactobacillus plantarum*. J Agricult Food Res 2025;21:101896. (doi: 10.1016/j.jafr.2025.101896)
27. Afrin S, Orr CH, Kuba S, et al. Microbiome profile and nutritional benefits of traditional overnight soaked cooked rice. Food Huma 2024;3:100410. (doi: 10.1016/j.foohum.2024.100410)
28. Lai QD, Doan NTT, Nguyen TH. Enhancing gamma aminobutyric acid synthesis from defatted rice bran extract by *Lactobacillus brevis* VTCC-B397: technical parameters analysis. Int J Food Sci Technol 2025;60(1): vvaf015. (doi: 10.1093/ijfood/vvaf015)