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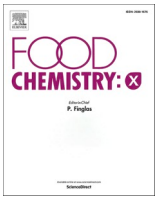

A simple and effective method to enhance the level of gamma-aminobutyric acid in Chinese yam tubers while preserving its original appearance

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ABSTRACT

Hot-air drying is an effective method to enhance the levels of gamma-aminobutyric acid (GABA) in edible tubers/tuberous roots. However, consumers prefer fresh food to processed food. Therefore, this study aims to develop an effective method to increase the GABA levels in the tubers of Chinese yam (CY tubers) and the tubers/tuberous roots of other plants while preserving its original appearance. Among nitrogen treatment (treatment under a nitrogen atmosphere), carbon dioxide (CO_2) treatment (treatment under a CO_2 atmosphere), vacuum treatment, and water immersion, CO_2 treatment was the most effective GABA-level-increasing method for CY tubers, with water immersion being more effective than nitrogen treatment and vacuum treatment. The GABA level in CY tubers treated with CO_2 for 72 h reached 1.25 ± 0.08 mg/g. CO_2 treatment and water immersion were also effective GABA-level-increasing methods for CY bulbils, potatoes, and lotus tubers, but they were less effective for carrots.

1. Introduction

The term functional food first appeared in a paper published in Nature in 1993, and it refers to food containing bioactive compounds such as probiotics, prebiotics, phenolic compounds, amino acids, fatty acids, phytosterols, fiber, and polyunsaturated fats (Badawy et al., 2023; Kouame et al., 2023; Swinbanks & O'Brien, 1993; Vanin, de Carvalho, Dos Santos Garcia, & Yoshida, 2023). Therefore, due to its bioactivities (immune-boosting, antioxidant, antibacterial, anti-inflammatory, antihypertensive, antianxiety, antidiabetic, antiobesity, and anticarcinogenic activities (Bello-Perez & Flores-Silva, 2023; Gouvarchinghaleh et al., 2023; Mittal, Mishra, Sharma, & Purohit, 2024; Shi, Jin, Wu, Zhu, & Cao, 2024)) and a growing awareness of the link between diet and health (Palachum, Klangbud, & Chisti, 2023), functional food has become increasingly popular. As a result, the size of the global market for functional food is expected to rise substantially from about 174.75 billion U.S. dollars in 2019 to over 275.77 billion U.S. dollars in 2025 (Mazumdar et al., 2023).

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that has some therapeutic effects including antihypertensive, antidiabetic, antianxiety, antihyperlipidemic, and anti-insomnia effects (Jitpakdee, Kantachote, Kanzaki, & Nitoda, 2021; Men et al., 2019; Minervini, Bilancia, Siragusa, Gobbetti, & Caponio, 2009; Sukegawa, Kokawa, & Kitamura, 2021). Therefore, the consumption of GABA-containing functional food is beneficial to human health (Inoue et al., 2003; Okada et al., 2000).

GABA is naturally present in grains, fruits, and vegetables, but its naturally occurring levels are very low (< 80 μg/g dry weight) (Pandey, Mettu, Mishra, Ashokkumar, & Martin, 2021). On the other hand, the use of chemically synthesized GABA as a food additive is prohibited in

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some countries (Abedin et al., 2024). Thus, GABA-enriched food has attracted considerable attention. The levels of GABA in cereals are usually increased through germination (Ding et al., 2016; Liu, Zhou, Wu, Chen, & Shu, 2022; Sukegawa et al., 2021; Wu, Li, Li, & Tan, 2022), and the GABA levels in dairy products (Ghazanfari, Falah, Yazdi, Behbahani, & Vasiee, 2024; Hurtado-Romero, Del Toro-Barbosa, Gradilla-Hernández, Garcia-Amezquita, & García-Cayuela, 2021; Jitpakdee et al., 2021; Minervini et al., 2009) are often increased through fermentation (Langa et al., 2024), which has also been used to increase the GABA levels in jujube (Men et al., 2019), drunken crab (Li, Li, Wan, Wang, & Zhou, 2022), and even kimchi beverage (Kwon, Kim, & Lee, 2025). Genome editing technique also used to enhance the GABA level in vegetable (Sakthivel et al., 2025). Meanwhile, the GABA levels in edible tubers/tuberous roots can be increased by sun drying or hot-air drying (Wang et al., 2022). Consumers generally prefer fresh food to processed food. Thus, this study aims to develop an effective method to enhance the GABA levels in edible tubers/tuberous roots such as fresh Chinese yam (CY) tubers while preserving its original appearance.

2. Materials and methods

2.1. Materials

CY tubers and CY bulbils were harvested from the same field in Wenxian County (Jiaozuo City, Henan Province, China). Potatoes, lotus tubers, carrots, and Chinese radishes were purchased from a supermarket in Zhengzhou City (Henan Province, China). Two commercial Chinese yam powder were purchased online, produced by Jiaozuo Lvzhou Huaiyao Biotechnology Co., Ltd. One is 100 % pure original powder, and the other one is 100 % pure original particle.

Deuterium oxide (D_2O, 99.9 %, Sigma Aldrich), methanol-d_4 (CD_3OD, 99.8 %, Cambridge Isotope Laboratories), and 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid sodium salt (TSP, 98 %, Cambridge Isotope Laboratories) were purchased from Tenglong Weibo Technology Co., Ltd. (China). Monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (China).

2.2. Sample preparation

CY tubers were categorized into different groups according to their processing methods (Table 1). The purpose of the steam cooking was to inactivate the glutamate decarboxylase (GAD) enzyme and remove the interfering effects of different levels of the enzyme on the different GABA levels in different groups of CY tubers, and the purpose of the hot-air drying was to improve sample extraction and weighing accuracy. HAD CY tubers were peeled, cut into pieces (1–3 mm), and dried with hot air (50 °C) for 48–72 h until their weights were stable, while SC-HAD CY tubers were cooked with steam for approximately 30 min (until they were easy to penetrate with a chopstick), peeled, cut into pieces (1–3 mm), and dried with hot air (50 °C) for 48–72 h until their weights were stable. On the other hand, CT-SC-HAD, NT-SC-HAD, WI-SC-HAD, and VT-SC-HAD CY tubers were treated under a 100 % carbon dioxide (CO_2) atmosphere, treated under a 100 % nitrogen atmosphere, immersed in water, and treated under vacuum conditions (each treatment was performed at room temperature for 96 h), respectively, before they were cooked with steam for approximately 30 min (until they were easy to penetrate with a chopstick), peeled, cut into pieces (1–3 mm), and dried with hot air (50 °C) for 48–72 h until their weights were stable. The results obtained for CT-SC-HAD, NT-SC-HAD, VT-SC-HAD, and WI-SC-HAD CY tubers were compared with those obtained for SC-HAD CY tubers to determine the effective GABA-level-increasing methods for CY tubers. On the other hand, they were compared with the results obtained for HAD CY tubers (Wang et al., 2022) to determine the effective CY processing methods. Dried CY tubers were powdered using an electric pulverizer and then passed through a 60-mesh sieve. Each group consists of 10 samples.

Table 1
Processing methods for fresh CY tubers.

Samples	Sample groups	Processing methods[a]		
		Treatment methods[a]	Cooking methods[b]	Drying methods after cooking[c]
CY tubers	SC-HAD	/	St	H
	CT-SC-HAD	carbon dioxide	St	H
	HAD	/	/	H
	NT-SC-HAD	nitrogen	St	H
	VT-SC-HAD	vacuum	St	H
	WI-SC-HAD	water	St	H

[a] /: no treatment; carbon dioxide: treatment under 1 atm and 100 % CO_2 atmosphere (CT); nitrogen: treatment under 1 atm and 100 % nitrogen atmosphere (NT); water: immersion in water (WI); and vacuum: treatment under a pressure ≤ -0.095 Mpa (VT). Each treatment was performed at room temperature for 96 h.
[b] /: no cooking; and St: cooking with steam (SC).
[c] H: drying with hot air (50 °C) (HAD).

2.3. Nuclear magnetic resonance (NMR) analysis

2.3.1. Sample preparation

Sample preparation for multivariate statistical analysis: A powdered-CY-tuber sample (50 mg) was added to a mixture of a 0.05 M phosphate buffer (0.4 mL, NaH_2PO_4 and Na_2HPO_4 in D_2O) and methanol-d_4 (0.4 mL). Then, the sample-containing mixture was vortexed for 5 min and centrifuged at 10,000 rpm for 5 min. The supernatant (550 μL) was transferred into a 5 mm NMR tube and used for NMR analysis.

Sample preparation for quantitative NMR analysis: A pulverized-CY-tuber sample (50 mg) was added to a mixture of a 0.05 M phosphate buffer (0.15 mL, NaH_2PO_4 and Na_2HPO_4 in D_2O) and methanol-d_4 (0.15 mL). Then, the sample-containing mixture was vortexed for 2 min and centrifuged at 10,000 rpm for 5 min, and the supernatant was transferred into a 5 mm NMR tube. The extraction procedure was repeated three times, resulting in a total of four rounds of extraction. Before NMR analysis, 20 μL of an internal standard solution was added into each NMR tube.

2.3.2. Preparation of an internal standard solution

TSP (21.5 mg) was dissolved in D_2O (10.0 mL) to obtain an internal standard solution with a 2.15 mg/mL concentration.

2.3.3. NMR analysis

Collection of NMR data for multivariate statistical analysis: Each ^1H NMR spectrum was collected by using a 400 MHz 400-MR NMR system (Agilent Technologies, USA) to perform the PRESAT solvent suppression method with 64 scans at 298 K. Fourier transformation was performed after 0.3 Hz exponential line-broadening. All ^1H NMR spectra were manually phased, baseline corrected, and calibrated (δ_H $CD_3OD = 3.31$) by using MestReNova software (version 10.0.1, Mestrelabs Research SL, Spain). The δ 0.50–9.50 region was included in equidistant bucketing with a 0.02 ppm bucket width. On the other hand, the δ 3.28–3.34 and δ 4.66–5.10 regions were excluded because they contained the residual signals of CHD_2OD and HDO, respectively. The peak integral of each bucketed region was normalized prior to an analysis of the NMR data.

Collection of NMR data for quantitative analysis: The parameters for data acquisition were as follows: the number of scans was 64 (generating 32 K data points), the spectral width was 4807 Hz, the acquisition time was 6.82 s, and the relaxation delay was 25 s. The exponential window function with 0.3 Hz line broadening was used for data processing. Phase and baseline corrections were performed manually using

MestReNova software (version 10.0.1, Mestrelabs Research SL, Spain). Chemical shifts were referenced to the signal of the internal standard (TSP; δ_H 0.00). After "GSD" was selected as the peak-picking method for quantitative NMR analysis, peaks for integration (the peak at δ_H 0.00 for TSP and the peaks at δ_H 2.32, 2.30, 2.28 for GABA) were picked, and "peaks" was selected as the peak-integral calculation method.

2.3.4. Statistic analysis

Multivariate data analysis was performed using SIMCA-P 14.0 software (Umetrics Inc., Sweden). Principal component analysis (PCA) was applied to unit-variance data to obtain an overview of the data set. Orthogonal partial least squares discriminant analysis (OPLS-DA) was applied to Pareto-scaled data (as the x-matrix) and group information (as the y-matrix) to investigate differences among samples.

3. Results and discussion

3.1. Identification of the metabolites in CY tubers

Metabolite assignment (Table S1) was performed according to the findings reported in our previous studies (Han et al., 2023; Wang et al., 2021), and differential metabolites were identified by performing multivariate statistical analysis on NMR-based metabolomic data. PCA was first applied to obtain an overview of the separation among CY tubers belonging to different groups. The PCA score plot shown in Fig. 1 demonstrated that hot-air-dried and CO_2-treated CY tubers were separated from CY tubers belonging to the other groups (steam-cooked, water-immersed, vacuum-treated, and N_2-treated CY tubers), indicating the distinct metabolites of the two groups of CY tubers. Moreover, the OPLS-DA score plots shown in Figs. 2A–2E demonstrated the good separation between steam-cooked CY tubers and CY tubers belonging to the other groups. The differential metabolites (VIP values >1.0) between steam cooked CY tubers and CY tubers belonging to the other groups were identified according to the loading column plots shown in Figs. 3A–3E. Permutation tests were performed to validate the OPLS-DA models with 200 permutations (Fig. S1).

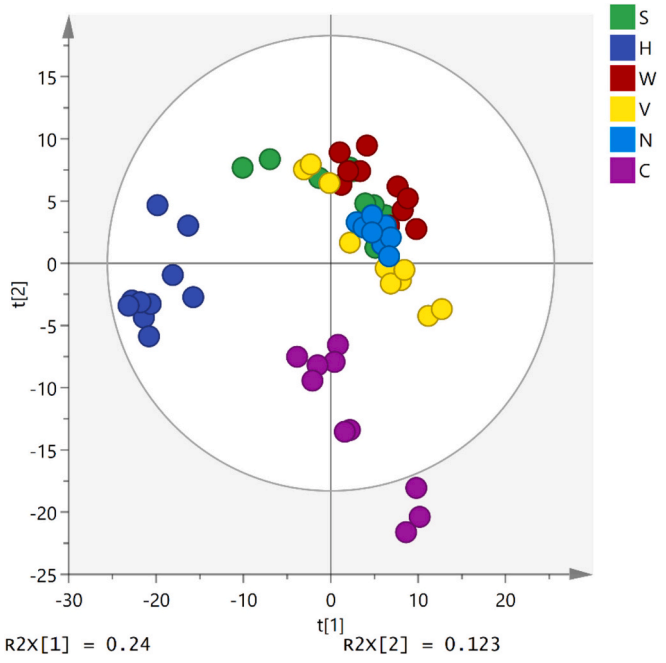

Fig. 1. A PCA score plot showing the separation of CY tubers belonging to different groups. S: SC-HAD CY tubers; C: CT-SC-HAD CY tubers; H: HAD CY tubers; N: NT-SC-HAD CY tubers; V: VT-SC-HAD CY tubers; W: WI-SC-HAD CY tubers.

The loading column plots showed that the GABA levels in hot-air-dried, CO_2-treated, and water-immersed CY tubers were higher than those in N_2-treated and vacuum-treated CY tubers. Moreover, the levels of different metabolites in hot-air-dried CY tubers were mostly higher than those in steam-cooked CY tubers (Fig. 3B), indicating that hot-air drying alone was the best processing method for CY tubers. In contrast to hot-air-dried, CO_2-treated, N_2-treated, and vacuum-treated CY tubers, water-immersed CY tubers showed decreased levels of saccharides, suggesting that they were suitable for people with diabetes. In addition, the glutamate level in CO_2-treated CY tubers was lower than that in steam-cooked CY tubers, indicating the conversion of glutamate into GABA (Wang et al., 2022).

CT: CO2 treated; NT: nitrogen treated; VT: vacuum treated; WI: water immersion treated; SC: steam cooked; HAD: hot air dried.

3.2. Relative levels of metabolites in CY tubers belonging to different groups

The levels of different metabolites in CY tubers belonging to different groups are demonstrated in Fig. 4. The levels of GABA in hot-air-dried, CO_2-treated, and water-immersed CY tubers were higher than those in steam-cooked, N_2-treated, and vacuum-treated CY tubers. The peak integrals of differential metabolites relative to the peak integral of the internal standard (TSP) are shown in Table 2 for a more accurate assessment. The GABA levels in hot-air-dried and CO_2-treated CY tubers were higher than those in CY tubers belonging to the other groups. Water-immersed CY tubers had a moderate GABA level, while the GABA levels in N_2-treated and vacuum-treated CY tubers were not significantly different from that in steam-cooked CY tubers. Compared with steam-cooked CY tubers, CO_2-treated CY tubers showed a significantly increased glucose level but a similar sucrose level. On the other hand, compared with steam-cooked CY tubers, hot-air-dried CY tubers showed increased levels for almost all metabolites. These results elucidated the principle behind the processing methods recommended by the Chinese Pharmacopeia for CY (peel, or water immersion, and then drying) (Chinese Pharmacopoeia Commission, 2020). Processed CY can be used as a material for Chinese medicine, while untreated CY can be used as food. Nitrogen treatment and vacuum treatment had no significant effect on the levels of metabolites other than alanine.

3.3. The relationship between the GABA level and CO_2 treatment time for CT-SC-HAD CY tubers

Fresh CY tubers were treated under a CO_2 atmosphere for 24, 48, 72, 96, or 120 h to determine the optimal CO_2 treatment time for CO_2-treated CY tubers. The GABA levels in the CO_2-treated CY tubers with different CO_2 treatment periods were estimated by a quantitative ^{1}H NMR method described in our previous work (Huang et al., 2023), and the results are shown in Table 3.

Table 3 shows that the GABA level in CO_2-treated CY tubers enhances as the CO_2 treatment time increases from 24 h to 72 h. However, the GABA level in the CY tubers decreased when the CO_2 treatment time increased from 72 h to 120 h. The GABA level in CO_2-treated CY tubers with 72 h CO_2 treatment reached 1.25 mg/g, which was slightly lower than that reported in our previous study (Huang et al., 2023) for hot-air-dried yam tubers (1.33 mg/g as determined by HPLC or 1.55 mg/g as determined by quantitative ^{1}H NMR).

3.4. Application of CY-tuber processing methods to CY bulbils and other edible tubers/tuberous roots

CY bulbils, potatoes, lotus tubers, and carrots were treated in the same way as CY tubers (Table 1). Similar to the results obtained for CY tubers, those obtained for CY bulbils showed that the levels of GABA in hot air-dried, CO_2-treated, and water-immersed CY bulbils were higher than those in N_2-treated and vacuum-treated CY bulbils (Figs. S2–S4).

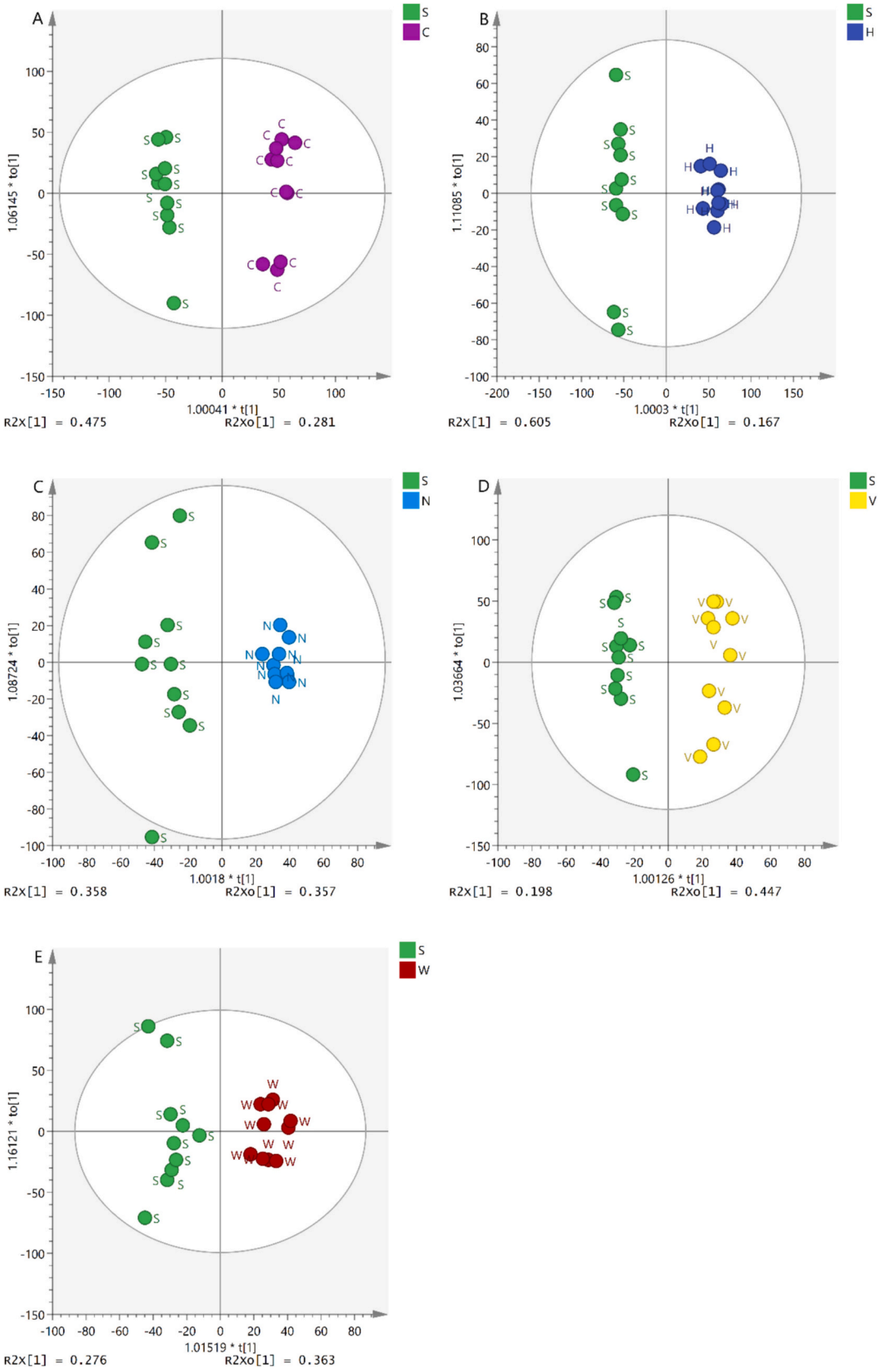

Fig. 2. OPLS-DA score plots showing the separation between: A, SC-HAD (S) vs. CT-SC-HAD (C) CY tubers; B, SC-HAD (S) vs. HAD CY (H) tubers; C, SC-HAD (S) vs. NT-SC-HAD (N) CY tubers; D, SC-HAD (S) vs. VT-SC-HAD (V) CY tubers; and E, SC-HAD (S) vs. WI-SC-HAD (W) CY tubers.

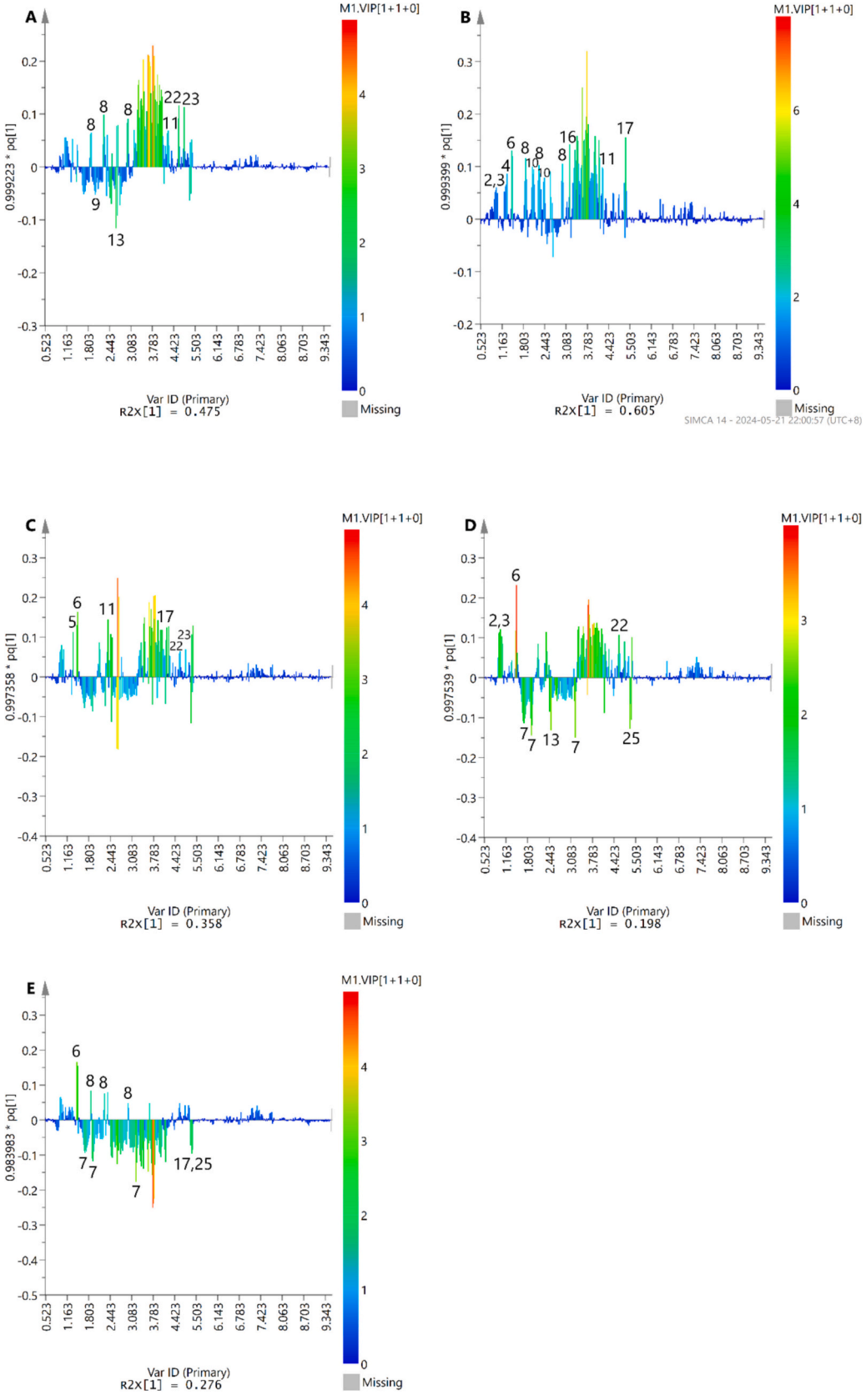

Fig. 3. Loading column plots showing metabolite-level comparisons for: A, SC-HAD (S) vs. CT-SC-HAD (C) CY tubers; B, SC-HAD (S) vs. HAD CY (H) tubers; C, SC-HAD (S) vs. NT-SC-HAD (N) CY tubers; D, SC-HAD (S) vs. VT-SC-HAD (V) CY tubers; and E, SC-HAD (S) vs. WI-SC-HAD (W) CY tubers. Metabolites: 2, isoleucine; 3, valine; 4, threonine; 5, 2-hydroxyisobutyrate; 6, alanine; 7, arginine; 8, GABA; 9, glutamate; 11, malate; 13, citrate; 17, sucrose; 22, β-D-glucose; 23, α-D-glucose; and 25, allantoin.

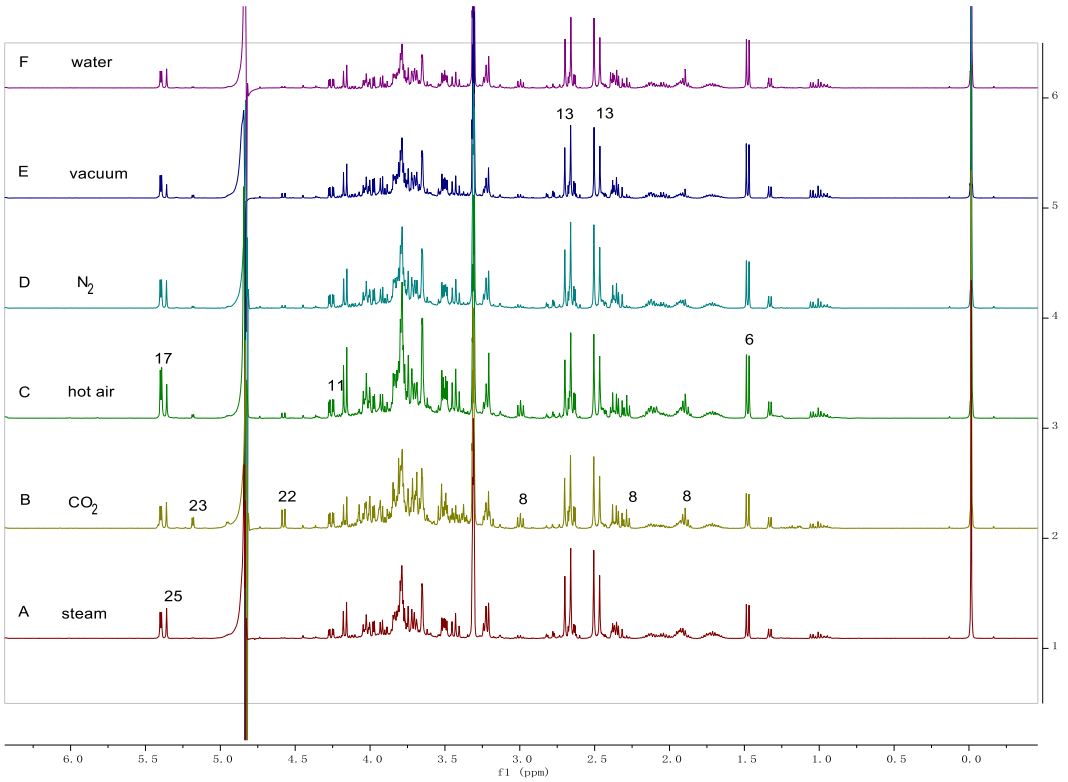

Fig. 4. ^{1}H NMR spectra of (A) SC-HAD, (B) CT-SC-HAD, (C) HAD, (D) NT-SC-HAD, (E) VT-SC-HAD, and (F) WI-SC-HAD CY tubers. Metabolites: 6, alanine; 8, GABA; 11, malate; 13, citrate; 17, sucrose; 22, β-D-glucose; 23, α-D-glucose; and 25, allantoin.

Table 2
Peak integrals of different metabolites relative to the peak integral of TSP (means ± standard deviations, $n = 10$).

Metabolites	Samples (integral)					
	SC-HAD	CT-SC-HAD	HAD	NT-SC-HAD	VT-SC-HAD	WI-SC-HAD
TSP(IS)	9.0	9.0	9.0	9.0	9.0	9.0
alanine	1.86 ± 0.03	1.94 ± 0.05	3.49 ± 0.06	2.70 ± 0.06	3.13 ± 0.12	2.69 ± 0.10
GABA[a]	0.25 ± 0.03	1.22 ± 0.06	1.57 ± 0.09	0.31 ± 0.03	0.39 ± 0.02	0.73 ± 0.04
Malate	0.90 ± 0.02	1.50 ± 0.06	1.88 ± 0.10	1.34 ± 0.04	1.10 ± 0.04	0.85 ± 0.08
Citrate[b]	5.69 ± 0.12	4.94 ± 0.17	6.05 ± 0.34	5.82 ± 0.16	5.29 ± 0.32	4.78 ± 0.32
Sucrose	1.75 ± 0.04	1.79 ± 0.04	3.39 ± 0.11	2.05 ± 0.05	1.79 ± 0.09	1.24 ± 0.06
β-D-glucose	0.058 ± 0.004	1.05 ± 0.03	0.30 ± 0.01	0.20 ± 0.01	0.33 ± 0.02	0.13 ± 0.01
α-D-glucose	0.024 ± 0.010	0.63 ± 0.02	0.21 ± 0.01	0.11 ± 0.00	0.19 ± 0.01	0.06 ± 0.01
allantoin	0.95 ± 0.04	0.86 ± 0.11	1.18 ± 0.08	0.89 ± 0.02	0.49 ± 0.07	0.60 ± 0.03

[a] The peak-integral-calculation region was the δ 2.96–3.02 region.
[b] The peak-integral-calculation region was the δ 2.46–2.54 region.

Table 3
GABA levels in CT-SC-HAD CY tubers with different CO_2 treatment periods (means ± standard deviations, $n = 3$).

Time	24 h	48 h	72 h	96 h	120 h
GABA (mg/g)	0.65 ± 0.00	1.00 ± 0.10	1.25 ± 0.08	1.14 ± 0.06	1.09 ± 0.01

Therefore, CO_2 treatment and water immersion were suitable GABA-level-increasing methods for CY bulbils, which are difficult to peel and slice. Large CY bulbils are utilized as seeds or food, but small CY bulbils are usually discarded because they have less economic value (Kim, Lee, Kim, Lee, & Lee, 2003; Teng et al., 2012). Therefore, an efficient processing method is beneficial to avoid the wastage of CY bulbils. CO_2 treatment, hot-air drying, and water immersion were also effective GABA-level-increasing methods for potatoes and lotus tubers, but they were less effective for carrots (Fig. S5-S7).

3.5. GABA level in commercial Chinese yam products

There are a variety of Chinese yam products available on the market, such as Chinese powder, Chinese yam chips, Chinese yam noodles, Chinese yam Tanghulu, Chinese yam cookies, mixed nut balls with Chinese yam and so on. Among these products, only Chinese yam powder is made entirely from Chinese yam (ingredients list). We determined the GABA content in two commercial Chinese yam powder and the results are listed in Table 4.

The two Chinese yam powders have different GABA contents due to different treatment methods. The original powder was dried by hot air and original particle was lyophilization. Although the color, mouthfeel, and dispersibility in water of lyophilization product are better than those of the hot air-dried product, the hot air-dried product possesses higher GABA level. This result is consistent with our previous study (Wang et al., 2022).

Table 4
GABA levels in commercial Chinese yam powder (means ± standard deviations, $n = 3$).

Name	Original powder	Original particle
GABA (mg/g)	1.07 ± 0.04	0.30 ± 0.02

4. Conclusion

PCA and OPLS-DA showed that the GABA levels in hot-air-dried (HAD), CO_2-treated (CT-SC-HAD), and water-immersed (WI-SC-HAD) CY tubers were higher than those in nitrogen-treated (NT-SC-HAD) and vacuum-treated (VT-SC-HAD) CY tubers. The GABA level in CO_2-treated CY tubers with 72 h CO_2 treatment reached 1.25 mg/g. Moreover, CO_2-treated CY tubers demonstrated the highest glucose level, while water-immersed CY tubers exhibited the lowest glucose level, elucidating the principle behind the standard CY processing methods described in the Chinese Pharmacopeia (water immersion, slicing, and then drying). CO_2 treatment and water immersion were also effective GABA-level-increasing methods for CY bulbils, potatoes, and lotus tubers, but they were less effective for carrots. Both the CO_2 treatment and water immersion methods do not alter the appearance of the food. Consumers can process the food using different methods such as steaming, boiling, frying, and baking, just as they would with fresh food. Moreover, the food processed by these methods cannot be adulterated. All these advantages indicate that the CO_2 treatment and water immersion are two useful food processing methods.

CRediT authorship contribution statement

Qiang Wang: Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Zhuo Chen:** Validation, Investigation, Data curation. **Xiqiang Gao:** Validation, Investigation. **Hongde Xu:** Investigation. **Yung-Yi Cheng:** Investigation. **Shuangyan Liu:** Validation. **Wei Wang:** Investigation. **Yuwei Zhang:** Investigation. **Dian Meng:** Investigation. **Yinuo Wang:** Investigation. **Shixiu Liao:** Supervision, Methodology. **Chengping Xie:** Writing – review & editing, Project administration. **Yanli Wang:** Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102379.

Data availability

Data will be made available on request.

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