國立臺灣海洋大學食品科學系碩士班

專題討論書面報告

發酵食品 GABA 的產生及前柱衍生化定量

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目錄

摘要

γ-胺基丁酸（GABA）為麩胺酸在麩胺酸脫羧酶（GAD）催化下所生成之非蛋白胺基酸，具有降血壓、調節中樞神經興奮、促進睡眠與維持神經訊號穩定等多重生理效益。發酵基質中的 GABA 生成量深受微生物群落組成、菌間交互作用、基質可利用性、環境酸鹼值等因素所影響。特別是具有 GAD 活性的乳酸菌（LAB）與芽孢桿菌（Bacillus spp.）在發酵過程中逐漸成為優勢族群，其透過 GAD 系統將麩胺酸轉換為 GABA。然而，發酵食品的基質極其複雜，GABA 含量常偏低且易受共存胺類、胜肽、游離胺基酸等物質干擾；傳統比色法或一般 UV 檢測難以達到足夠的靈敏度與選擇性。因此，近年食品化學領域逐漸以「前柱衍生化結合高效液相層析（HPLC）」取代傳統分析方法。OPA 與 DABS-Cl 分別代表兩大衍生化策略：OPA-螢光衍生化具高靈敏度但衍生物穩定性有限，而 DABS-Cl 形成之共軛芳香胺衍生物則具備優異的穩定性與適用於多種基質的可行性。本研究綜整微生物生態動態與前柱衍生化-HPLC 分析技術，並建立可用於品質監測與機能性驗證的準研究等級分析架構，期能為後續功能性食品開發提供完整科學依據。

Quantitative Analysis of GABA Production and Pro-column Derivatization in Fermented Foods

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Outline

Abstract

γ-Aminobutyric acid (GABA) is a non-protein amino acid produced from glutamate through the catalytic action of glutamate decarboxylase (GAD). It exerts multiple physiological benefits, including blood pressure reduction, modulation of central nervous system excitability, promotion of sleep, and stabilization of neural signaling. The amount of GABA generated in fermentation substrates is strongly influenced by factors such as microbial community composition, interspecies interactions, substrate availability, environmental pH. In particular, lactic acid bacteria (LAB) and *Bacillus* spp., which frequently become dominant during fermentation due to their inherent GAD activity, convert glutamate into GABA through the GAD system. However, the complex composition of fermented-food matrices often results in low GABA levels and substantial interference from coexisting amines, peptides, and free amino acids. Traditional colorimetric assays or general UV detection often fail to deliver sufficient sensitivity and selectivity. Consequently, in recent years, the food chemistry field has shifted toward “pre-column derivatization coupled with high-performance liquid chromatography (HPLC)” as a replacement for conventional analytical techniques. OPA and DABS-Cl represent two major derivatization strategies: OPA-based fluorescent derivatization provides high sensitivity but limited derivative stability, whereas DABS-Cl forms conjugated aromatic amine derivatives with excellent stability and broad matrix applicability. The goal is to establish a research-grade analytical framework for quality monitoring and functional verification, thereby providing a comprehensive scientific foundation for future functional food development.

1. 前言

γ-胺基丁酸（GABA）是一種由麩胺酸脫羧生成的非蛋白胺基酸，具有降血壓、安神及改善神經傳導等多種生理功效，因此廣泛應用於功能性食品開發。近年研究指出，發酵食品是外源性 GABA 的主要來源之一，而其生成量受到發酵微生物群落結構與代謝活性的高度影響。尤其乳酸菌與芽孢桿菌等具麩胺酸脫羧酶（GAD）活性的菌株，能有效促進 GABA 的生合成，使微生物動態組成成為決定發酵品質的重要因素。

然而，發酵基質中 GABA 的含量低、干擾物多，傳統定量方法往往缺乏靈敏度與專一性。因此，建立一套能準確評估 GABA 生成的分析流程，成為研究與產業的關鍵課題。相關研究表明，結合前柱衍生化與高效液相層析（HPLC）可顯著提升分析靈敏度與選擇性。常用試劑如 o-phthalaldehyde（OPA）與 DABS-Cl 能將氨基酸轉化為具螢光或吸光特性的衍生物，搭配 FLD、UV 或 DAD 偵測均可達到穩定分離與準確定量。

因此本研究在解析發酵過程中微生物群落與 GABA 合成之關聯，並建立適用於複雜食品基質的高準確度 HPLC 分析方法，以此法作為功能性發酵食品的品質評估與代謝機制研究提供科學依據。

2. 探討發酵食品中 GABA 的產生

引用 Utama et al.（2025）之研究，印尼蝦醬（terasi）為典型的自發性高鹽發酵食品，其微生物生態變化可視為 GABA 生成機制的最佳模型。研究以 16S rRNA 高通量定序解析發酵 0、3、7、14 日菌相變化，並同步以 OPA-HPLC 定量麩胺酸與 GABA。由圖二可得知，發酵初期由巨大球菌與弧菌占優勢，發酵中期後乳酸菌與芽孢桿菌成為主要菌群，圖四說明發酵物中 GABA 的濃度自第 7 日開始大幅上升，並在第 14 日達高峰，圖五結果顯示具 GAD 基因活性菌株相對豐度與 GABA 上升呈強正相關。此結果明確支持「乳酸菌優勢化 → GAD 系統啟動 → GABA 累積」的因果鏈，並顯示微生物動態可作為預測發酵食品 GABA 功能性的核心指標。此結果證實 GABA 的生成主要受菌群代謝結構驅動，微生物多樣性與代謝活性共同影響最終發酵品質。

3. 柱前衍生化協助 HPLC 定量 GABA

本章全面整合 OPA 與 DABS-Cl 兩大衍生化策略之化學反應機制、儀器分析特性與色層分離理論，以重構 GABA 於複雜食品基質中的高精度定量方法學。OPA 與 DABS-Cl 雖同屬前柱衍生化試劑，但二者在反應物構型、衍生物穩定性、色譜行為、偵測器適配性等面向具有根本差異，因此理解其反應機理與最佳化條件是建立高準確度 HPLC 定量的關鍵。

A. 使用 OPA 作為衍生化試劑進行衍生化

OPA（o-phthalaldehyde）衍生化反應需胺基（如 GABA 的 α-NH_2）與一種巰基化合物（如 β-mercaptoethanol）共存，形成具有強螢光訊號之異吲哚（isoindole）結構。反應機制為，OPA 的兩個醛基與胺基形成 Schiff base，巰基化合物進入環化反應生成異吲哚螢光團，產物具 $\lambda_{ex} \approx 340$ nm、$\lambda_{em} \approx 450$ nm 之高強度螢光。然而，OPA 衍生物化學穩定性不佳，易於數十分鐘內開始降解，因此分析時間需精準控制並避免樣品放置過久。

在 C18 色層柱中，OPA-GABA 衍生物因其芳香異吲哚結構而具有中度保留行為，保留時間易受流動相中乙腈比例影響，故梯度系統通常由較低比例乙腈起始，以確保與其他胺類化合物之間具足夠分離度。

OPA 系統適用於低濃度分析，其優勢為「極高靈敏度」，惟缺點為衍生物不穩定、須立即進樣、基質干擾大時可能出現峰形扭曲。

B. 使用 DABS-Cl 作為衍生化試劑進行衍生化

DABS-Cl（4-dimethylaminoazobenzene-4´-sulfonyl chloride）屬於芳香磺醯氯化合物，與胺基反應後形成高度共軛的偶氮芳香胺結構，使其在 463 nm 具強吸光性。反應機構可分為，胺基進攻磺醯氯形成取代反應、產物形成穩定的芳香磺醯胺鍵，由於共軛系統延伸，衍生物具有高穩定性且可於室溫下保存 24 小時不降解。

DABS-Cl 衍生化的穩定性遠優於 OPA，因此更適合用於多基質（茶飲、固體食品、發酵食品）分析，其色層行為於 C18 管柱中呈現較強保留能力，通常需較高乙腈才可達成良好洗脫。然而，反應時間較長（約 30 分鐘）且對 pH 敏感，因此需控制反應環境。

DABS-Cl 系統的最大特色為「衍生物高度穩定、適用於多樣食品基質」，缺點為衍生化步驟較繁複。

4. 結論

本研究透過整合微生物生態、GAD 相關代謝路徑與前柱衍生化 HPLC 定量技術，重構了發酵食品中 GABA 累積之核心機制。從微生物層面觀察，乳酸菌（LAB）與芽孢桿菌（Bacillus spp.）的優勢化 與 GAD 基因相對豐度呈現強正相關，證實發酵過程中微生物演替為驅動 GABA 生合成的重要決定因子。另一方面，Glu → GABA 的代謝轉換與環境 pH、基質組成及菌株耐酸特性密切相關，顯示發酵條件 在調節代謝通量中扮演關鍵角色。

在化學分析上，本研究亦比較 OPA 與 DABS-Cl 兩大衍生化系統之差異。OPA 衍生化具備螢光高靈敏度 之優勢，適合低濃度分析；然衍生物不穩定且易受基質干擾，需於短時間內完成進樣。DABS-Cl 衍生化 雖偵測靈敏度略低，但產物高度穩定、峰形一致性佳，尤其適用於含多種干擾物之食品基質。結合 C18 色層理論（k'、Rs、α）與方法驗證結果（LOD、LOQ、RSD、Recovery），本研究成功建立一套適用於 發酵食品之高準確度 GABA 定量流程。

本成果不僅能補強功能性發酵食品之品質控管，亦可提供未來 starter culture 設計、發酵條件優化、多體學整合（multi-omics）、代謝通量分析及生物活性驗證的重要基礎，為後續學術與產業應用奠定 關鍵科學依據。

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Table. 1. Modification of scheme of elution gradient for HPLC analysis (Le et al.,2020)

Reaction time (min)	Glu			GABA		
	Glu PA/IS PA	CV%	RSD%	GABA PA/IS PA	CV%	RSD%
1	1.115 ± 0.041	3.7	7.0	1.563 ± 0.037	2.4	7.0
2	1.121 ± 0.038	3.4		1.515 ± 0.043	2.8	
3	1.119 ± 0.035	3.1		1.452 ± 0.076	5.2	
4	1.114 ± 0.036	3.2		1.385 ± 0.072	5.2	

PA peak area; *IS* internal standard; *CV* coefficient of variation; *RSD* relative standard deviation

Table. 2. Modification of scheme of elution gradient for HPLC analysis (Le et al.,2020)

1st modification of gradient			2nd modification of gradient		
Time (min)	Solvent A (%)	Solvent B (%)	Time (min)	Solvent A (%)	Solvent B (%)
0	90	10	0	80	20
27	0	100	24	0	100
37	0	100	34	0	100
42	100	0	39	100	0
57	100	0	53	100	0

Table. 3. Linear ranges, linear equation, R2value, LODs, and LOQs of Glu and GABA in 75%EtOH and water measured by HPLC method (Le et al.,2020)

Solvent	Glu					GABA				
	Linear ranges (mg/L)	Linear equation	R^2	LOD (mg/L)	LOQ (mg/L)	Linear ranges (mg/L)	Linear equation	R^2	LOD (mg/L)	LOQ (mg/L)
Water	1–100	$y = 0.0087 \times x$	0.9957	0.75	1.5	1–60	$y = 0.0185 \times x$	0.999	0.78	1.57
75% EtOH	1–100	$y = 0.0056 \times x$	0.9961	0.36	0.71	1–60	$y = 0.0188 \times x$	0.9866	0.51	1.02

Table. 4. Glu and GABA content in fermented foods extract (75%EtOH and water with 4%SSA) measured by HPLC and spectrophotometric methods (Le et al.,2020)

Products	HPLC method				Spectrophotometric method	
	Glu (mg/g DW)		GABA (mg/g DW)		GABA (mg/g DW)	
	75% EtOH	Water	75% EtOH	Water	75% EtOH	Water
Vegetable products						
Fermented Bonbon	0.40 ± 0.01	< LOQ	0.62 ± 0.02	< LOQ	19.38 ± 0.78	7.9 ± 0.7
Fermented green mustard	0.92 ± 0.17	0.77 ± 0.11	0.77 ± 0.12	0.61 ± 0.07	17.70 ± 0.73	10.4 ± 0.6
Fermented coconut shoot	0.38 ± 0.08	< LOQ	< LOQ	< LOQ	16.30 ± 0.60	4.80 ± 1.50
Fermented leek	0.15 ± 0.01	0.15 ± 0.01	0.24 ± 0.01	0.19 ± 0.01	5.95 ± 0.40	3.89 ± 0.17
Fermented garlic	6.73 ± 0.85	6.51 ± 0.21	< LOQ	< LOQ	16.50 ± 1.20	10.10 ± 0.40
Fermented radish	17.56 ± 0.64	13.62 ± 0.37	1.38 ± 0.01	1.26 ± 0.02	25.40 ± 1.26	17.40 ± 1.53
Fermented sweet and sour eggplant	4.33 ± 0.16	3.92 ± 0.17	0.99 ± 0.04	0.87 ± 0.01	6.74 ± 0.37	3.94 ± 0.15
Fermented eggplant	19.85 ± 1.16	16.64 ± 0.27	2.94 ± 0.06	2.75 ± 0.06	18.13 ± 1.10	10.41 ± 0.20
Soy derivates						
Fermented tofu	13.69 ± 0.95	13.77 ± 0.99	1.83 ± 0.07	2.40 ± 0.08	98.50 ± 2.52	53.76 ± 1.37
Fermented soybean	5.33 ± 0.11	5.07 ± 0.13	1.38 ± 0.07	1.43 ± 0.02	23.91 ± 0.40	15.85 ± 0.46
Soya sauce	111 ± 8.19	99.13 ± 8.83	0.22 ± 0.00	0.22 ± 0.01	7.38 ± 0.74	4.69 ± 0.39
Animal and fish-based products						
Fermented milk	0.57 ± 0.05	0.51 ± 0.05	0.08 ± 0.00	0.08 ± 0.01	2.31 ± 0.09	1.56 ± 0.09
Fermented pork	3.97 ± 0.61	5.10 ± 0.43	0.52 ± 0.06	0.54 ± 0.03	15.90 ± 1.78	13.78 ± 0.23
Fermented sour shrimp	3.14 ± 0.45	2.98 ± 0.07	1.73 ± 0.07	1.79 ± 0.02	19.16 ± 0.94	12.57 ± 0.27
Fermented anchovies	15.64 ± 0.51	14.73 ± 0.57	6.21 ± 0.30	5.86 ± 0.20	35.24 ± 3.96	25.08 ± 0.88
Fish sauce	37.85 ± 1.15	36.87 ± 1.50	16.29 ± 0.53	16.71 ± 0.64	127.01 ± 3.52	91.97 ± 1.09

Table. 5. The results of six consecutive injections of 50μg/mL mixed standard working solution. (Xiao et al.,2024)

Serial Number	The Peak Area of DABS-Theanine (mAU)	RSD (%)	The Peak Area of DABS-GABA (mAU)	RSD (%)
1	1,447,947	0.08	3,695,706	0.10
2	1,449,504		3,694,736	
3	1,446,784		3,685,472	
4	1,447,279		3,693,983	
5	1,449,319		3,691,117	
6	1,447,828		3,694,924	

Table. 6. The detection result of 50μg/mL mixed standard solution within 24h. (Xiao et al.,2024)

Time (h)	The Peak Area of DABS-Theanine (mAU)	RSD (%)	The Peak Area of DABS-GABS (mAU)	RSD (%)
0	1,447,947	0.37	3,695,706	0.49
2	1,447,828		3,694,924	
4	1,441,699		3,647,233	
6	1,447,330		3,679,871	
8	1,449,575		3,659,602	
12	1,434,174		3,681,925	
24	1,443,504		3,681,633	

Table. 7. Recoveries and RSDs of the target analytes spiked in different sample matrices. (Xiao et al.,2024)

Sample	Additive Amount (mg/kg)		Recovery (%)						RSD (%)
Crisps	50	Theanine	95.18	96.68	102.18	99.08	99.18	100.28	2.54
		GABA	101.97	99.77	95.77	98.27	101.87	100.97	2.41
	100	Theanine	97.39	97.69	96.24	103.19	98.34	100.34	2.54
		GABA	98.13	100.78	101.13	98.08	99.88	102.13	1.65
	200	Theanine	101.12	99.05	100.35	99.67	100.62	96.10	1.82
		GABA	102.54	101.72	102.07	103.24	99.67	98.57	1.78
Coated Tofu	50	Theanine	97.70	100.40	101.70	95.60	98.60	101.10	2.34
		GABA	100.50	97.90	99.10	98.50	101.60	98.60	1.41
	100	Theanine	97.40	96.10	103.65	102.25	101.70	99.70	2.94
		GABA	97.50	99.80	94.40	103.90	102.65	103.05	3.70
	200	Theanine	101.45	95.23	98.15	101.15	102.18	93.95	3.53
		GABA	102.85	101.50	97.33	102.13	99.90	100.95	1.95

Sample	Additive Amount (mg/kg)		Recovery (%)						RSD (%)
GABA Candy	50	Theanine	99.20	94.30	97.90	103.00	96.60	103.70	3.70
		GABA	103.87	100.47	98.17	99.87	102.67	99.47	2.11
	100	Theanine	98.85	98.75	98.55	97.95	100.90	97.10	1.28
		GABA	94.53	99.63	101.33	94.93	98.83	100.63	2.96
	200	Theanine	102.43	97.60	99.25	97.93	98.43	98.75	1.76
		GABA	94.59	100.29	96.14	103.37	94.82	95.87	3.62
Green Tea Beverage	50	Theanine	99.70	95.30	102.40	99.50	103.40	99.20	2.84
		GABA	101.60	96.10	98.50	96.90	103.80	98.80	2.93
	100	Theanine	99.90	98.30	99.35	99.00	96.90	94.40	2.08
		GABA	98.40	98.65	97.90	102.40	97.45	98.75	1.79
	200	Theanine	95.75	95.75	97.70	95.78	95.25	103.15	3.11
		GABA	99.13	98.08	103.58	98.10	100.70	101.45	2.15
Matcha Cookie	50	Theanine	98.98	97.08	97.88	101.68	100.08	98.88	1.64
		GABA	98.82	94.02	98.92	98.42	95.82	96.92	2.01
	100	Theanine	94.09	96.69	99.64	97.64	95.89	99.74	2.26
		GABA	94.76	98.01	97.06	99.46	99.71	96.56	1.92
	200	Theanine	99.27	100.77	99.35	99.75	101.27	98.30	1.08
		GABA	98.30	96.23	99.80	99.88	94.85	98.75	2.07
Solid Beverage	50	Theanine	99.52	98.82	96.22	96.42	102.82	102.22	2.81
		GABA	97.77	96.27	97.57	95.37	99.17	100.97	2.05
	100	Theanine	98.26	101.16	94.81	96.81	96.51	101.51	2.73
		GABA	94.73	99.83	96.53	94.48	94.98	98.03	2.22
	200	Theanine	97.10	100.48	96.73	99.60	97.13	96.85	1.66
		GABA	103.79	100.49	97.27	97.22	95.92	96.89	3.02
Theanine and GABA Mixed drink	50	Theanine	95.97	103.67	98.27	101.57	102.77	102.57	3.00
		GABA	101.08	100.38	99.88	101.38	98.68	99.68	0.99
	100	Theanine	102.83	96.83	101.83	96.58	94.13	103.43	3.93
		GABA	97.74	99.44	94.14	99.64	98.79	102.69	2.83
	200	Theanine	102.67	101.59	95.29	99.99	99.34	97.59	2.70
		GABA	101.15	97.70	98.20	99.47	96.90	98.47	1.51

Table. 8. The results of actual samples. (Xiao et al.,2024)

Samples		Content (mg/kg)						Average Value (mg/kg)	RSD (%)
Theanine and GABA bonbon	Theanine GABA	1330.10 7088.40	1313.75 7012.10	1336.80 6528.75	1309.65 6687.05	1264.05 6563.10	1318.40 6587.90	1312.13 6744.55	1.96 3.61
Theanine and GABA gummy candy	Theanine GABA	1093.10 5282.20	1100.20 5134.90	1118.15 5299.75	1110.80 5211.30	1100.90 5129.65	1130.15 5158.25	1108.88 5202.68	1.23 1.43
Theanine and GABA mixed drink	Theanine GABA	768.75 5778.50	768.20 5744.25	774.40 5800.25	785.20 5817.75	762.90 5785.55	773.85 5826.35	772.22 5792.11	0.99 0.51
Black tea beverage	Theanine GABA	375.60 22.90	366.85 22.50	370.20 22.60	371.95 23.30	366.80 23.35	373.65 23.25	370.84 22.98	0.97 1.62
Solid beverage	Theanine GABA	302.40 93.50	302.55 90.65	312.75 91.90	305.70 94.35	307.55 91.70	314.00 91.50	307.49 92.27	1.62 1.50

Table. 9. Comparison of the introduced method with other methods. (Xiao et al.,2024)

Analytes	Samples	LOD (mg/kg)	LOQ (mg/kg)	Linear Range (μg/mL)	Derivatization Reagent	Derivatization Time (min)	Separation Time (min)	RSD (%)	Method	Ref.
Theanine	Green tea	3×10^{-3}	0.01	0.02–1.0	-	-	16	3.0–10.5	GC-MS/MS	Gao et al. 2023 [15]
Theanine	Tea	9.8×10^{-2}	-	0.87–34.8	Formaldehyde and sodium borohydride	-	5	-	CE-ECL	Yi et al. 2017 [17]
Theanine	Tea	5.7×10^{-4}	1.88×10^{-3}	10–200	CBBC-Cl	6	14	2.24–4.85	HPLC-FLD	Wang et al. 2020 [18]
GABA	Alcoholic beverage	0.1–8.2	0.25–27.3	10–500	-	-	6	1.17–7.93	LC-MS/MS	Liu et al. 2023 [20]
GABA	Germinated brown rice	9.8×10^{-4}	-	5–60	OPA	2	20	-	HPLC-DAD	Cheng et al. 2014 [21]
GABA	Fermented soybean products	3	10	1–100	FMOC-Cl	5	45	0.56–4.21	HPLC-DAD	Zhuang et al. 2023 [22]
GABA	Plants foods and medicinal plants	9	-	2–1000	Dansyl-Cl	60	-	0.73–3.72	HPLC-DAD	Pencheva et al. 2022 [24]
Theanine and GABA	Tea	2.6 and 4.1	8.7 and 13.7	1.74–69.6 and 1.03–41.2	OPA and NAC	1	19 and 18	-	HPLC-FLD	Tu et al. 2012 [23]
Theanine and GABA	Tea	1.162×10^{-2} and 3.4×10^{-3}	-	-	DABS-Cl	10	20 and 40	-	HPLC-DAD	Syu et al. 2008 [34]
Theanine and GABA	Food	0.6 and 0.2	1.7 and 0.6	1–100	DABS-Cl	20	11 and 15	0.99–3.93	HPLC-DAD	This method

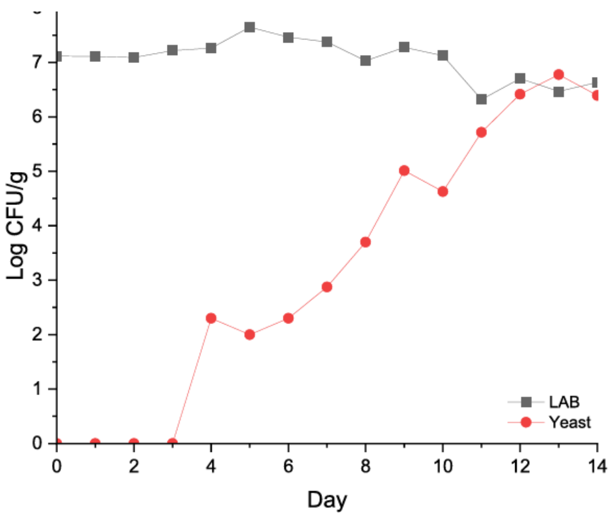

Fig. 1. LAB and Yeast growth during terasi spontaneous fermentation D0 until D14. (Utama et al.,2024/2025)

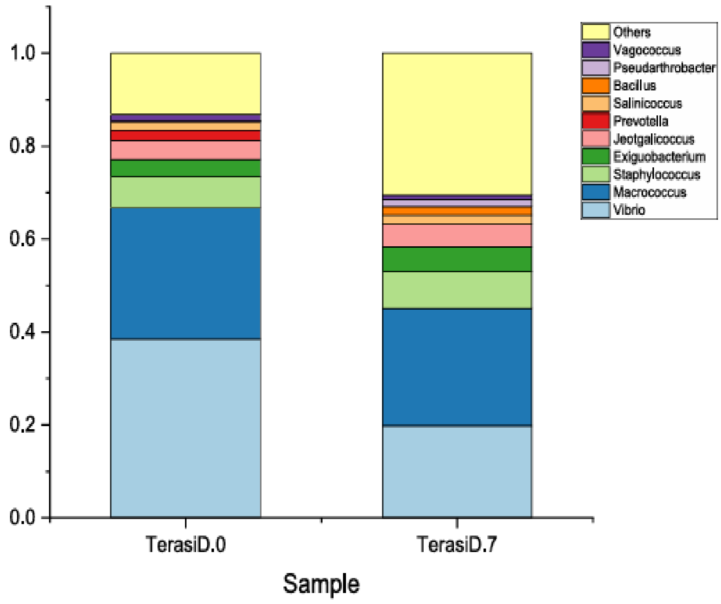

Fig. 2. The top 10 taxa relative abundance in genera level. (Utama et al.,2024/2025)

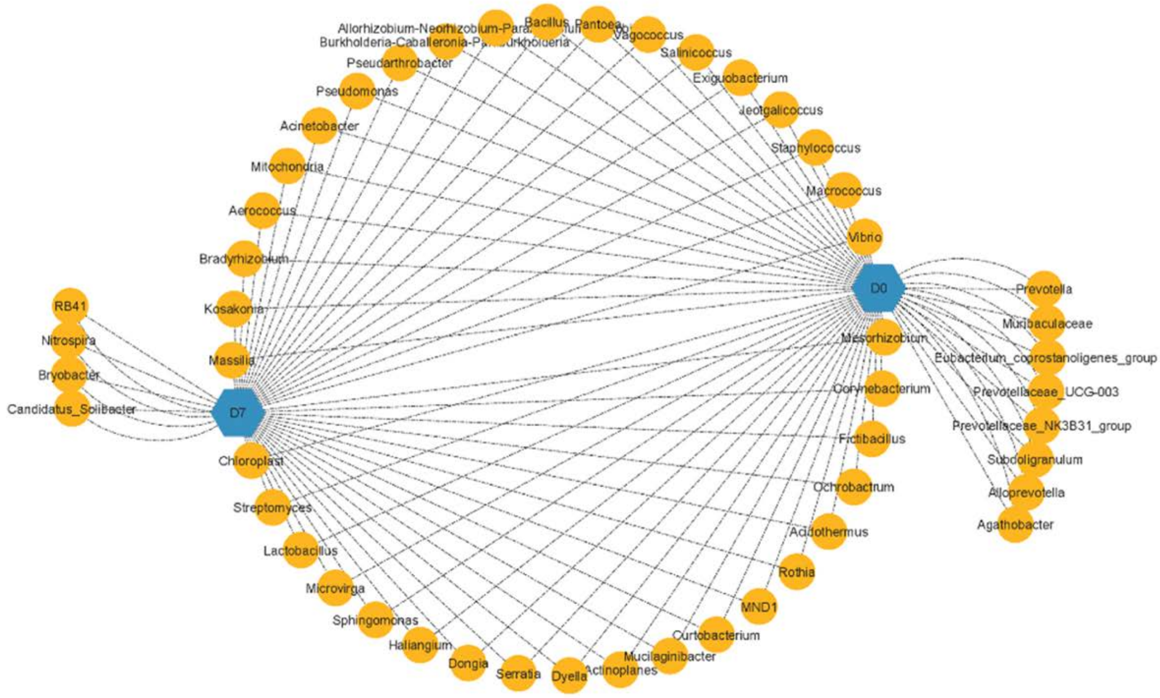

Fig. 3. Microbiome network analysis of core communities of bacteria during terasi spontaneous fermentation. (Utama et al.,2024/2025)

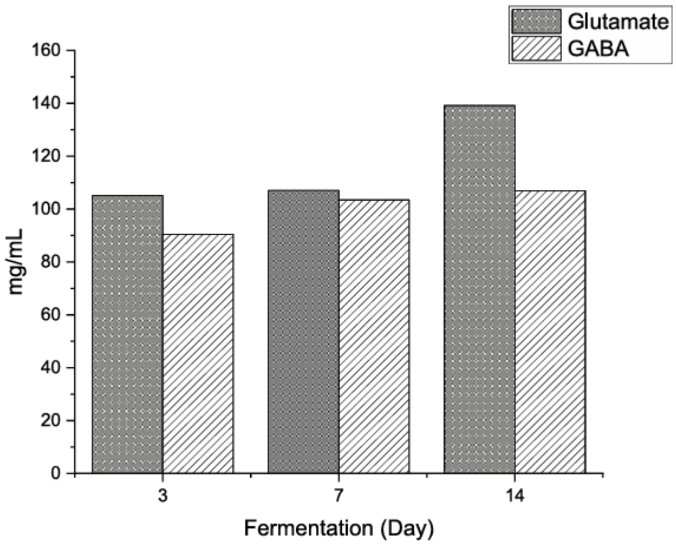

Fig. 4. Amount of glutamate and GABA during the spontaneous fermentation process of D3, D7, and D14. (Utama et al.,2024/2025)

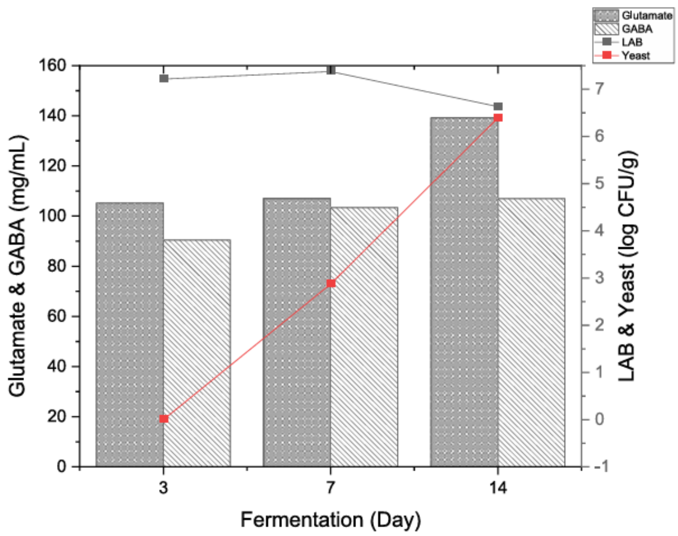

Fig. 5. The relationship curve between LAB growth and yeast and the amount of glutamate and GABA detected in D3, D7, and D14. (Utama et al.,2024/2025)

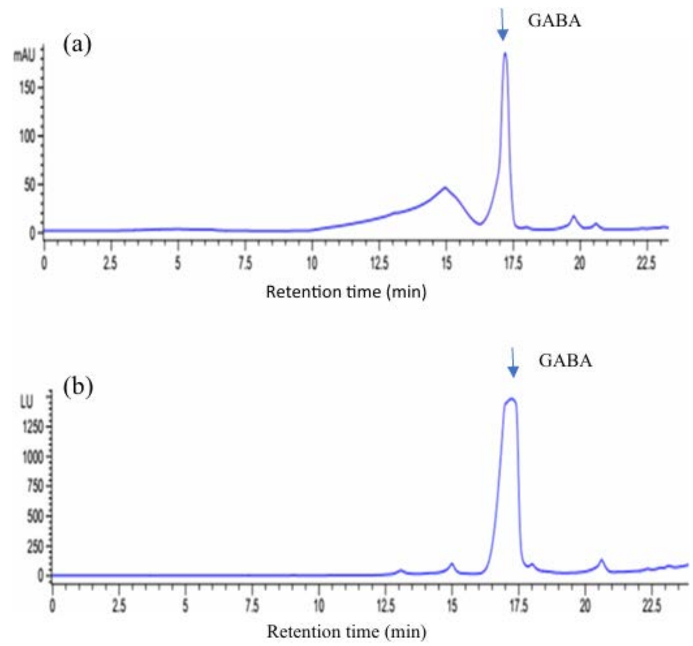

Fig. 6. Chromatograms showing the elution of derivatization of a GABA standard (500 ppm) detected with DAD at 338 nm (a) , FLD at Ex=340, Em=455 (b) (Le et al.,2020)

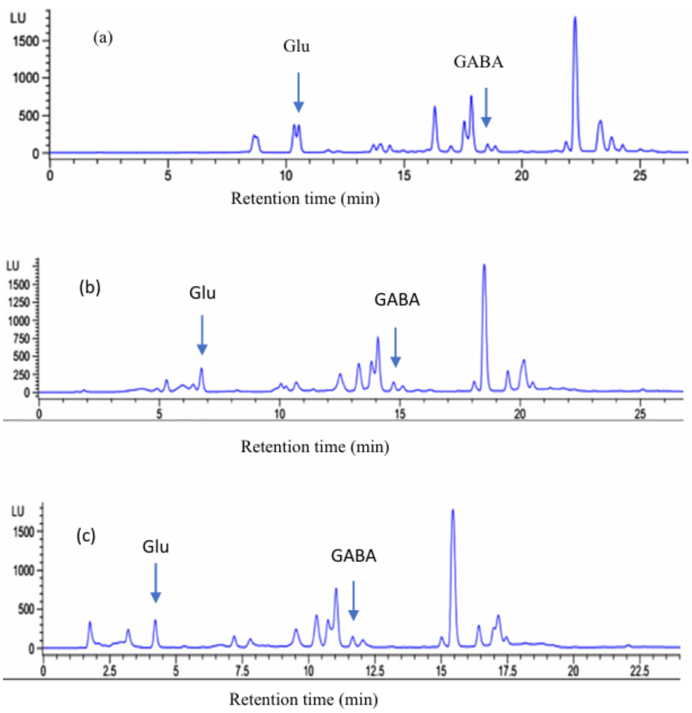

Fig. 7**. a** Gradient of the initial method. **b** Gradient of the 1st modification. **c** Gradient of the 2nd modification. (Le et al.,2020)

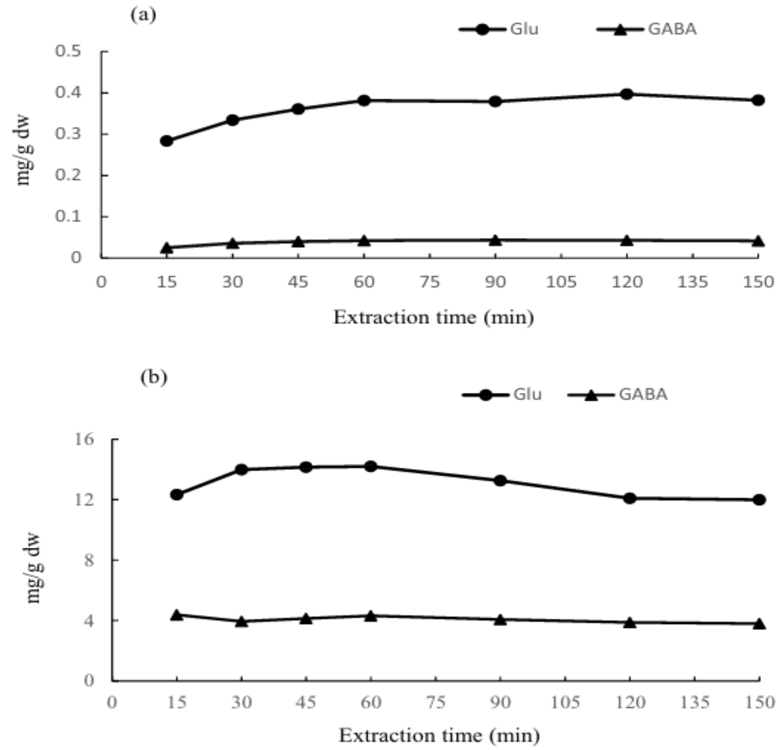

Fig. 8. Effect of extraction time on Glu and GABA content of soybean (a) and tempeh (b) with 75% EtOH measured by HPLC method. (Le et al.,2020)

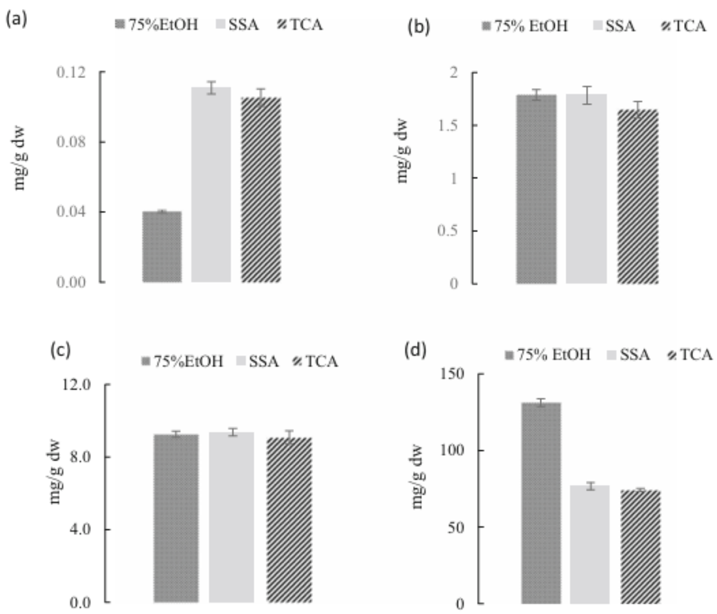

Fig. 9. The extraction conditions in the extraction of GABA from soybeans(a and b) and tempeh(c and d)by the HPLC method(a and c) and spectrophotometric method (b and d) (Le et al.,2020)

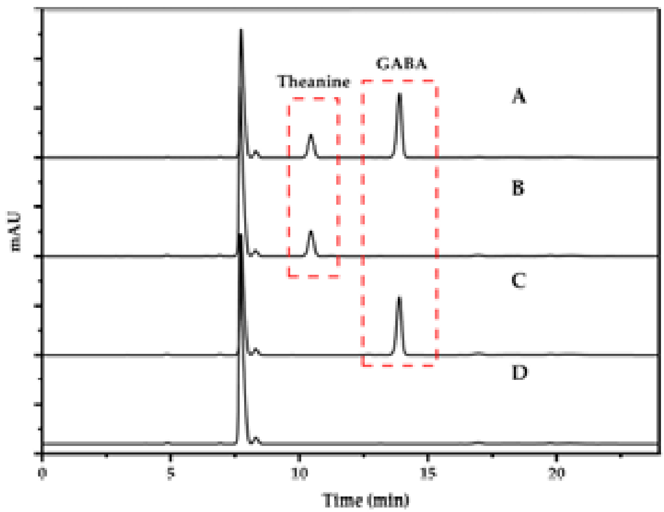

Fig. 10. Chromatograms: Theanine and GABA(A), Theanine(B), GABA(C), ultra-pure water(D) (Xiao et al.,2024)

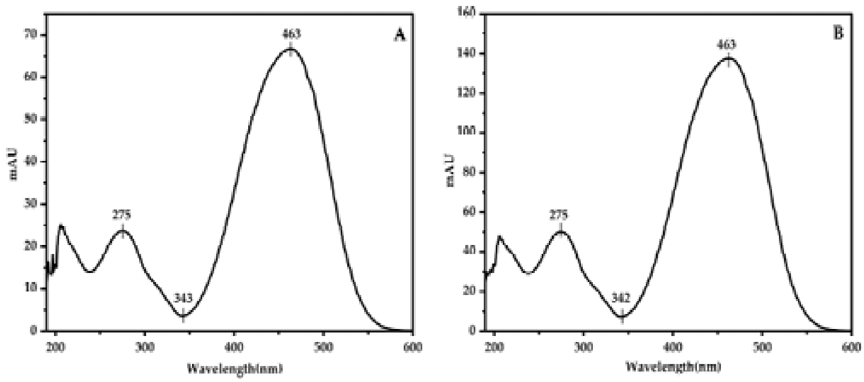

Fig. 11. The spectrum of the standard solution of DABA-Theanine (A) and DABA-GABA (B) (Xiao et al.,2024)

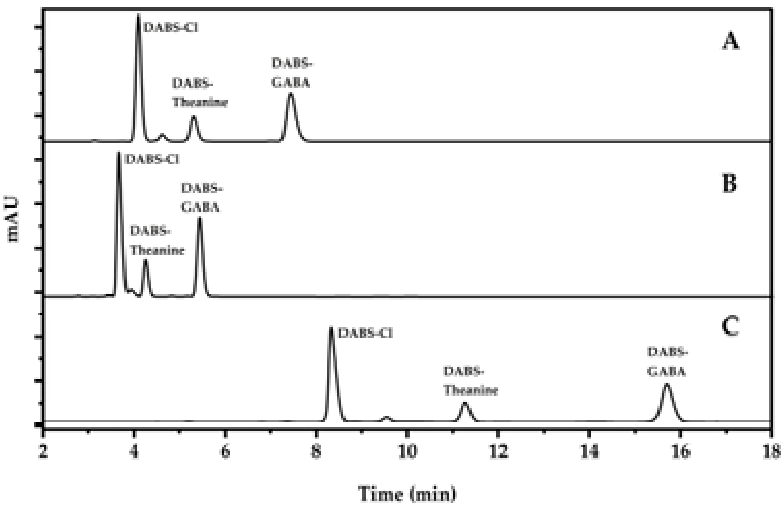

Fig. 12. Chromatograms of different chromatographic columns: Platisil ODS(A), Kinetex C18 100Å(B), Capcell Pak ADME-HR(C). (Xiao et al.,2024)

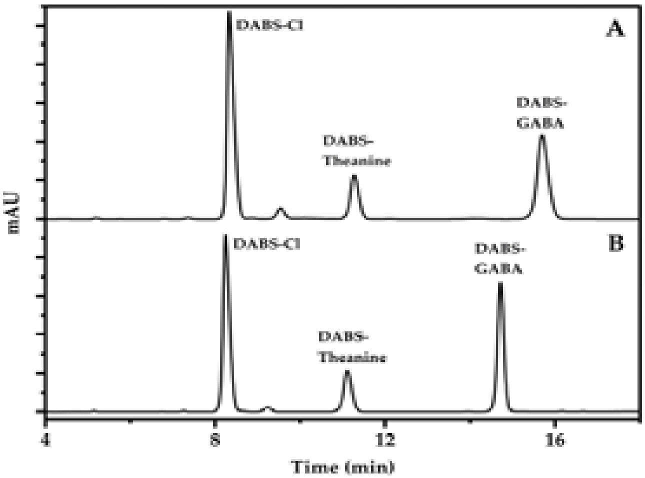

Fig. 13. Chromatograms of different mobile phase gradients : Isocratic(A), Gradient(B). (Xiao et al.,2024)

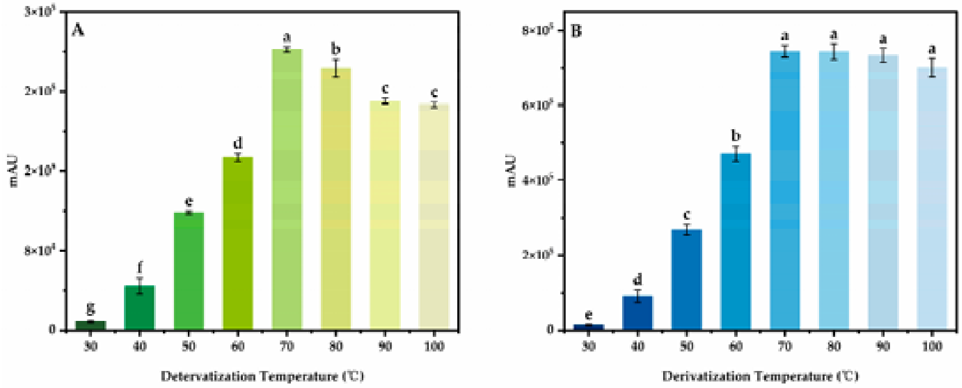

Fig. 14. derivatization temperature on peak area. (A)DABS-Theanine; (B)DABS-GABA. (Xiao et al.,2024)

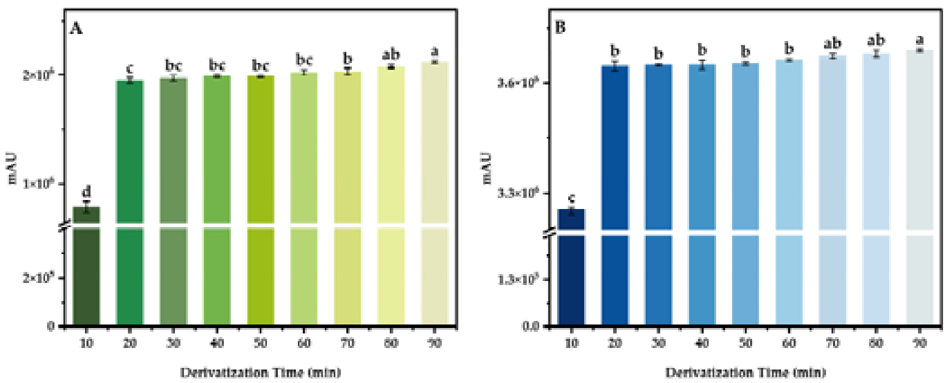

Fig. 15. derivatization time on the peak area. (A)DABS-Theanine; (B)DABS-GABA. (Xiao et al.,2024)

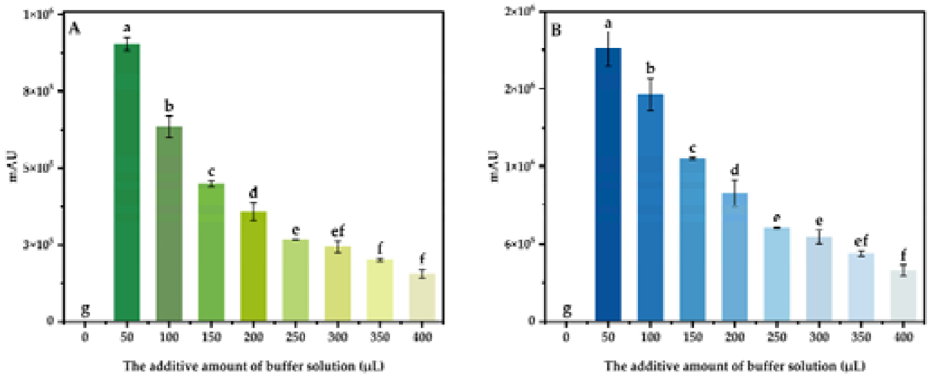

Fig. 16. different amount of buffer solution. (A)DABS-Theanine; (B)DABS-GABA. (Xiao et al.,2024)

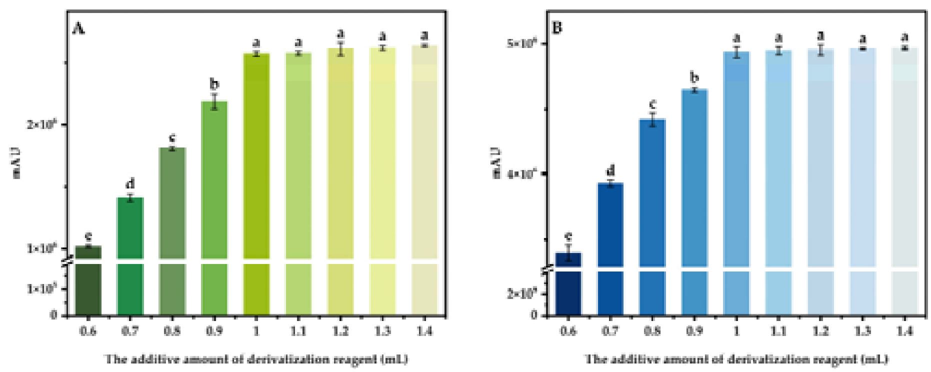

Fig. 17. different amount of derivatization reagent. (A)DABS-Theanine; (B)DABS-GABA. (Xiao et al.,2024)

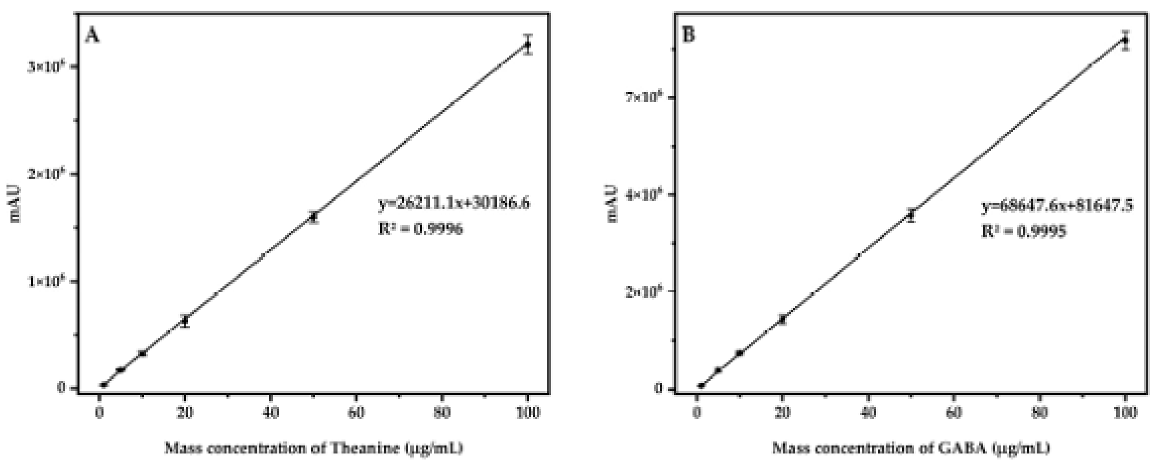

Fig. 18. Standard curve of (A)Theanine; (B)GABA. (Xiao et al.,2024)

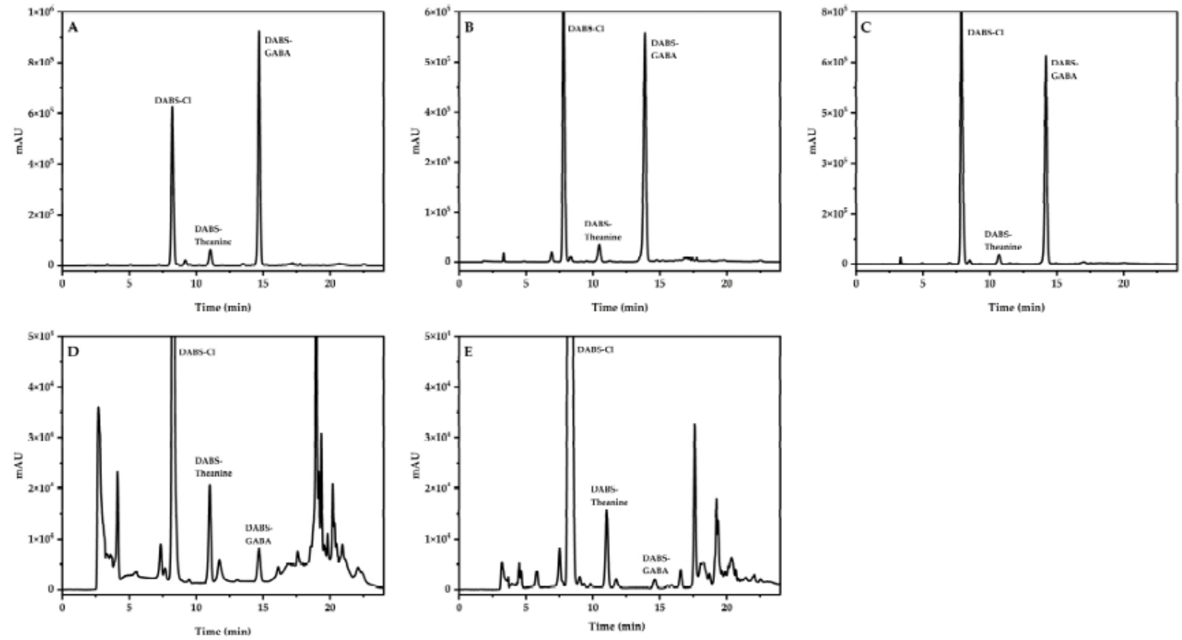

Fig. 19. Chromatograms of actual samples. ((A)Theanine and GABA bonbon; (B)Theanine and GABA gummy candy; (C)Theanine and GABA mixed drink; (D)Black tea beverage; (E)Solid beverage). (Xiao et al.,2024)