

Research Article

Study on the Enhancement of Hypoglycemic and Antioxidant Activities of Compound Herbal Tea through Solid-State Fermentation

¹Qing Liu, ¹Xiaowen Que, ¹Yueping Yang, ¹Jinghao Zhao, ²Tian Tian, ³Jiixin Wang, ⁴Zhaosen Fan, ⁵Hua Fang and ¹Yuanda Song

¹School of Agricultural Engineering and Food Science, Shandong University of Technology, China

²School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, China

³Management, Shandong Lejiake Food Co., Ltd., China

⁴Executive, Shandong Benon Biological Technology Co., Ltd., China

⁵Management, Shandong Hezhong Kangyuan Biotechnology Co., Ltd, China

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Corresponding Author:

Yuanda Song

School of Agricultural Engineering

and Food Science, Shandong

University of Technology, China

Email: ysong@sdut.edu.cn

Abstract: This study utilized *Aspergillus niger* KY2295 for the Solid-State Fermentation (SSF) of a compound tea made from *Cinnamomum cassia* bark, *Glycyrrhiza glabra*, *Morus abla* L. leaf, and *Crataegus oxyacantha* L. to enhance its hypoglycemic effects. The fermentation process was optimized using single-factor experiments and a Central Composite Design (CCD), with α -amylase and α -glucosidase inhibitory activities as evaluation indices. Following fermentation, increases were observed in active compounds, including total phenolics, flavonoids, polysaccharides, proteins, and free amino acids. In vitro antioxidant activities-2,2-Diphenyl-1-Picrylhydrazyl (DPPH), 2,2'-Azino-Bis (3-ethylbenzothiazoline-6-Sulfonic acid) (ABTS), and hydroxyl radical scavenging-were enhanced, contributing to increased enzyme inhibition. The optimal conditions (3.88% inoculum, 30.01°C, 73.50 h) yielded these two enzymes inhibition rates of 62.21±1.94 and 72.39±1.18%, respectively. DPPH, ABTS, and hydroxyl radical scavenging increased by 18.47, 47.55, and 20.22%, respectively. Furthermore, the inhibition rates of α -amylase and α -glucosidase rose by 43.37 and 54.84%. These results demonstrate that SSF significantly enhances the bioactive content, hypoglycemic and antioxidant activities of the herbal tea, suggesting potential for its development as a functional food.

Keywords: Compound Herbal Tea, Hypoglycemic Activity, Solid-state Fermentation, *Aspergillus niger*, Antioxidant Activity

Introduction

In recent years, the use of natural substances and foods as functional products for managing hyperglycemia has garnered significant attention. Unlike synthetic drugs, which often come with various side effects, natural products offer a safer alternative with fewer adverse effects (Xue *et al.*, 2023). Extensive research has elucidated that specific naturally occurring compounds exhibit significant hypoglycemic activity, thereby positioning them as promising candidates for the formulation of functional foods targeting glycemic control (Zhang *et al.*, 2022a; Ma *et al.*, 2022; Li *et al.*, 2023a).

Functional herbal teas have emerged as a promising category within this realm owing to their wide range of bioactive components, ease of consumption, and cultural

acceptance (Dujnič *et al.*, 2021; Malongane *et al.*, 2020). Herbal teas containing specific medicinal plants, such as cinnamon, licorice, mulberry leaf, and hawthorn, have been shown to exert beneficial effects on blood sugar levels (Zhang *et al.*, 2022b; Özcan *et al.*, 2017). These hypoglycemic effects are primarily attributed to the rich array of bioactive compounds in tea, such as polyphenols, flavonoids, saponins, organic acids, terpenes, polysaccharides, and volatile oils (Bai *et al.*, 2013). However, many of these compounds are bound within complex structures like cell walls and lignin, limiting their extractability through conventional methods and thereby reducing their bioavailability and efficacy.

SSF has emerged as an innovative approach for enhancing the bioavailability and bioactivity of active compounds in herbal products. Through microbial

enzymes such as cellulases, pectinases, and glucosidases, SSF degrades plant cell walls and releases bound bioactive compounds, including polyphenols, flavonoids, and saponins, which are associated with hypoglycemic and antioxidant effects. (Janarny and Gunathilake, 2020; Liu *et al.*, 2022a) . This process also hydrolyzes polysaccharides into smaller, more easily absorbed oligosaccharides, which may enhance hypoglycemic effects (Gnomou *et al.*, 2023). Moreover, SSF can generate novel bioactive metabolites, including organic acids, peptides, and bioactive lipids, which may further contribute to the overall biological activity of the fermented product. The use of *A. niger* in SSF has been reported to significantly increase the antioxidant and hypoglycemic activities of various substrates, demonstrating potential in both research and practical applications (Gnomou *et al.*, 2023; Huang *et al.*, 2019). Additionally, SSF offers practical advantages due to its mild operational conditions, high conversion efficiency, and simplified post-processing requirements, making it an accessible method for enhancing functional foods (Ng *et al.*, 2022).

Despite these advantages, research on the application of SSF to composite herbal teas, particularly with the aim of enhancing hypoglycemic effects, remains limited. This study addresses this gap by presenting a novel application of SSF using *A. niger* to increase the bioactivity of a composite hypoglycemic tea. The tea was formulated from four traditional herbs- *C. cassia* bark (cinnamon bark), *G. glabra* (licorice), *M. abla* L. leaf (mulberry leaf), and *C. oxyacantha* L. (hawthorn) – each historically recognized for their health benefits, particularly in blood glucose regulation. While prior studies have demonstrated the hypoglycemic and antioxidant potentials of these individual herbs, their combined effects, especially following fermentation, remain largely unexplored.

To address this gap, we optimized the fermentation conditions and analyzed the content of key bioactive components before and after fermentation. Our primary hypothesis was that fermentation would enhance the bioavailability and efficacy of these components, leading to improved *in vitro* hypoglycemic and antioxidant activities. Through the present study, we endeavor to underscore the potential of SSF as a means to markedly augment the functional attributes of herbal teas, offering insights into the development of safe and effective functional foods for blood glucose regulation.

Materials and Methods

Sample Preparation

C. cassia bark, *G. glabra*, *M. abla* L. leaf, and *C. oxyacantha* L. were purchased from Beijing Tongrentang pharmacy. The four herbs were weighed and mixed in an optimal proportion of 2:2:1:2, then freeze-dried. The

dried samples were finely ground, sieved through an 80-mesh screen to ensure uniform particle size, and subsequently stored under appropriate conditions for further use.

Strain Cultivation and Solid-State Fermentation

A. niger KY2295, provided by Shandong Hezhong Kangyuan Biotechnology Co., Ltd., was used due to its high enzyme activity in breaking down plant cell walls, which effectively releases bioactive compounds in herbal substrates. Potato Dextrose Agar (PDA) plates were employed for the cultivation of spores, which were incubated at 28 °C for a period of 3 days to facilitate sporulation. The resulting spore biomass was subsequently harvested into a sterile Erlenmeyer flask, rinsed with 100 mL of sterile distilled water, and mixed thoroughly using an ultrasonic homogenizer. The concentration of the spore suspension was standardized to 1×10^7 CFU/mL.

SSF was carried out as follows: Each 10 g of dried herbal mixture was sterilized at 121 °C for 15 min, cooled, mixed with 5 mL of sterile water, and inoculated with 4 mL of the prepared *A. niger* KY2295 spore suspension before incubation at 30°C.

Single-Factor Experiment for Fermentation Optimization

Based on previous research conducted in our lab, three key factors - spore inoculation amount, fermentation temperature, and time - were identified as key factors. For inoculation amounts, 2, 3, 4, 5, and 6% were tested; temperatures of 24, 26, 28, 30, and 32 °C; and times of 24, 48, 72, 96, and 120 h. The inhibition rates of α -amylase and α -glucosidase were used to evaluate each condition, with an unfermented sample as the blank control and acarbose as the positive control.

Central Composite Design (CCD) for Process Optimization

According to the single-factor results, spore inoculation amount, fermentation temperature, and fermentation duration were designated as independent variables within a Central Composite Design (CCD) framework to further refine the fermentation conditions. The inhibitory activities against these two enzymes served as the response variables, with the corresponding factors and levels detailed in Table (1).

Table 1: Independent variables and their corresponding coded levels in the central composite design framework

No. Independent variables	Coded levels	Symbol	
		Coded low	Coded high
1 Inoculation amount (%)	A	3 (-1)	5 (1)
2 Fermentation temperature (°C)	B	28 (-1)	32 (1)
3 Fermentation duration (h)	C	48 (-1)	96 (1)

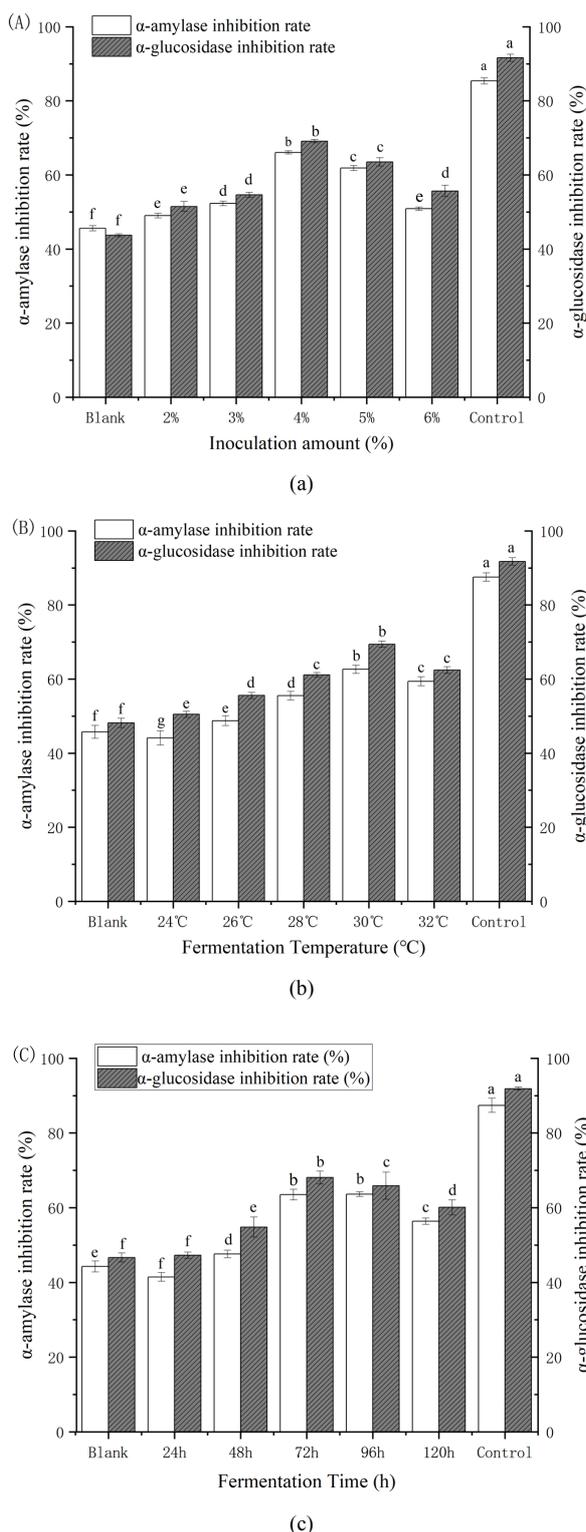


Fig. 1: The effect of inoculation amount, temperature, and duration on α -amylase and α -glucosidase inhibition. (a) Inoculation amount; (b) Fermentation temperature; (c) Fermentation time. The error bars denote the Standard Deviation (SD) derived from three independent biological samples. Statistically significant differences between values are indicated by distinct superscripts ($p < 0.05$)

Enzyme Inhibition Assays

The inhibition rates of α -amylase and α -glucosidase were determined using the methods previously established in our lab (Liu *et al.*, 2022b).

Determination of Bioactive Compounds

Quantification of total phenolic compounds was performed utilizing the Folin–Ciocalteu colorimetric assay, following the protocol outlined by Joshua *et al.* (2023). Total polysaccharide content was assessed via the phenol–sulfuric acid method, as described by Liu *et al.* (2022c), employing glucose as the standard reference. Total flavonoids were quantified using a previously described method, with slight modifications to adjust the standard curve concentration range to 20–500 $\mu\text{g/mL}$, ensuring alignment with the sample measurement range (Bautista-Hernández *et al.*, 2022). Protein quantification was carried out using the Coomassie Brilliant Blue G-250 dye-binding method (Chen *et al.*, 2018). Free amino acids were analyzed using the ninhydrin colorimetric method (Dong *et al.*, 2023).

Antioxidant Activity Assessment

DPPH, ABTS, and hydroxyl radical scavenging activities were determined as indicators of antioxidant activities. The DPPH radical scavenging activity was quantified according to a previously established protocol, as outlined in prior studies (Liu *et al.*, 2022a). The evaluation of ABTS radical scavenging activity was carried out as outlined by Wang and Wang (2023), whereas the determination of hydroxyl radical scavenging activity was conducted based on the method reported by Wen *et al.* (2023), with the reaction carried out in the dark at 37°C for 40 min as a modification.

Statistical Analysis

The data obtained from three independent experiments were subjected to statistical analysis using SPSS Statistics Software, Version 22.0 (SPSS, Inc., Chicago, IL, USA). Analysis was performed through one-way tests, with the results expressed as mean \pm SD. To assess the statistical significance of differences between means, a Student's t-test was applied, considering a p-value < 0.05 as indicative of statistical significance. Additionally, regression analysis of fermentation parameters was conducted utilizing Design-Expert 8.0.6 software.

Results

Effect of Inoculation Amount, Temperature, and Time on α -Amylase and α -Glucosidase Inhibition by Fermented Compound Tea

Figure (1) illustrates the influence of inoculation volume, fermentation temperature, and fermentation

duration on the inhibition rates of these two enzymes by fermented compound. In each bar plot, distinct letters positioned above the bars denote statistically significant differences ($p < 0.05$) among groups, highlighting the impact of each variable on enzyme inhibition. The two carbohydrate-degrading enzymes play crucial roles as key enzymes within the gastrointestinal system responsible for breaking down dietary carbohydrates into glucose, which is then absorbed into the bloodstream. Inhibiting these enzymes can help control postprandial blood glucose levels, making them critical indicators for evaluating hypoglycemic effects and potential diabetes relief (Pantidos *et al.*, 2014).

In Figure (1A), as the inoculation amount increased from 2-4%, the inhibition rates rose, peaking at 4 % with 66.07 % for α -amylase and 69.10% for α -glucosidase. Beyond this point, the rates declined, with the 6% inoculation amount showing 50.92 and 55.67% inhibition for these two carbohydrate-degrading enzymes, respectively. Figure (1B) shows that increasing the fermentation temperature from 24-32 °C resulted in higher inhibition rates, reaching a maximum at 30 °C with 62.63% for α -amylase and 69.40% for α -glucosidase. In Figure (1C), inhibition rates increased with fermentation time, peaking at 72 h with 63.50% for α -amylase and 68.07% for α -glucosidase. After 72 h, the rates decreased, with the 120 h fermentation period showing inhibition rates of 56.37 and 54.77% for these two enzymes, respectively.

Fermentation Optimization Using Central Composite Design

In this study, fermentation optimization was conducted using Central Composite Design (CCD), specifying the range for each independent variable. The independent variables included inoculation amount (A, %), fermentation temperature (B, °C), and fermentation duration (C, h). As outlined in Table (2), the CCD matrix.

The corresponding response values are presented. The experimental design included 20 runs, each representing a unique combination of independent variables, performed in random order. The responses measured were the inhibition rates of α -amylase (Response 1, %) and α -glucosidase (Response 2, %). The inhibition rates of these two enzymes in the herbal compound tea ranged from 47.16 to 65.69% and from 50.58 to 73.78%, respectively, as influenced by the fermentation parameters (Table 2).

The Response Surface Method Fitting Model

The CCD approach, incorporating three variables, was applied to identify the optimal combination of variables and corresponding response patterns. The data were analyzed through a multiple regression approach with a second-order polynomial model to explore the relationships between variables. The fitting results are presented in Tables (3-4). Both models, predicting the

inhibition rates of the two enzymes involved in starch and sugar breakdown, showed highly significant results ($p < 0.0001$). The values of lack of fit were 0.6437 and 0.1121, both higher than 0.05. The coefficient of determination (R^2) values for the two responses were 0.9634 and 0.9882, respectively. The resulting regression equations for the inhibition rates of the two enzymes are presented as follows:

$$E_{\alpha\text{-amylase inhibition rate}} = 63.43 - 1.16A + 0.3342B + 1.17C - 1.61AB - 1.90AC + 0.51BC - 3.76A^2 - 2.73B^2 - 5.25C^2 \quad (1)$$

$$E_{\alpha\text{-glucosidase inhibition rate}} = 72.59 - 0.4329A - 0.8242B - 0.2816C - 1.56AB - 1.27AC - 0.2562BC - 4.01A^2 - 6.48B^2 - 6.97C^2 \quad (2)$$

Equations (1-2), derived from the CCD approach, are used to predict the inhibition rates of α -amylase and α -glucosidase. Each equation includes three linear terms, three quadratic terms, three interaction terms and a constant.

Table 2: Central composite design matrix and corresponding response values for fermentation optimization

Std	Run	A (%)	B (°C)	C (h)	Response 1 (%)	Response 2 (%)
12	1	0	1.414	0	55.85	53.64
13	2	0	0	-1.414	47.16	54.43
5	3	-1	-1	1	53.91	56.85
7	4	-1	1	1	59.97	56.19
9	5	-1.414	0	0	53.82	61.90
6	6	1	-1	1	51.96	54.37
15	7	0	0	0	62.37	72.35
19	8	0	0	0	65.69	71.07
11	9	0	-1.414	0	54.48	56.26
20	10	0	0	0	61.62	73.78
16	11	0	0	0	63.41	72.74
18	12	0	0	0	65.53	72.68
2	13	1	-1	-1	52.25	58.19
4	14	1	1	-1	49.82	52.32
10	15	1.414	0	0	50.66	61.97
14	16	0	0	1.414	48.89	52.72
17	17	0	0	0	62.18	72.66
8	18	1	1	1	49.07	50.58
3	19	-1	1	-1	50.63	55.96
1	20	-1	-1	-1	49.11	52.49

A, Inoculation amount (%); B, Fermentation temperature (°C); C, Fermentation time (h); Response 1: Inhibition rate of α -amylase (%); Response 2: Inhibition rate of α -glucosidase (%).

Effect of Fermentation Parameters on the Inhibition of α -Amylase

As illustrated in Table (3), the interplay between inoculation quantity and fermentation period (AC), along with the quadratic term for inoculation amount (A^2), fermentation temperature (B^2), and fermentation time (C^2), exhibited remarkably significant effects on the α -amylase inhibition activity of the herbal tea ($p < 0.01$).

Additionally, A, C, and AB also contributed significantly to the inhibition rate ($p < 0.05$). The 3D response surface charts in Fig. (2A-C) illustrate how these parameters interact to influence α -amylase inhibition. In Fig. (2A), the inhibitory activity against α -amylase varied with changes in inoculation amount and fermentation temperature, increasing to a peak before declining. Figure (2B) shows that the inhibition rate also varied with inoculation volume and fermentation period, initially increasing and then decreasing after reaching a maximum. In Fig. (2C), the interaction between fermentation temperature and fermentation time is shown, with the inhibition rate rising to a peak before decreasing as both variables continued to increase. These inhibition rates are notably higher than those typically

observed in unfermented samples, indicating that the fermentation process significantly enhances the α -amylase inhibitory activity of the tea.

Effect of Fermentation Parameters on the Inhibition of α -Glucosidase

Table (4) reveals that the inhibition of α -glucosidase by the herbal compound tea is significantly influenced by the interaction between inoculation amount and fermentation temperature (AB), as well as the quadratic terms of the independent variables (A^2 , B^2 , C^2), all of which were highly significant ($p < 0.01$). Additionally, the linear term for fermentation temperature (B) and the interaction between fermentation time and inoculation amount (AC) were also significant ($p < 0.05$).

Table 3: Variance analysis for the fitting of the quadratic model on α -amylase inhibition efficiency;

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	694.16	9	77.13	29.21	< 0.0001	significant
A	18.36	1	18.36	6.95	0.0249	
B	1.53	1	1.53	0.5776	0.4648	
C	18.77	1	18.77	7.11	0.0237	
AB	20.8	1	20.8	7.88	0.0186	
AC	28.8	1	28.8	10.91	0.0080	
BC	2.08	1	2.08	0.7879	0.3956	
A^2	203.84	1	203.84	77.19	< 0.0001	
B^2	107.15	1	107.15	40.58	< 0.0001	
C^2	397.39	1	397.39	150.48	< 0.0001	
Residual	26.41	10	2.64			
Lack of Fit	10.94	5	2.19	0.7068	0.6437	not significant
Pure Error	15.47	5	3.09			
Cor Total	720.57	19				
	$R^2 = 0.9634$					

A, Inoculation amount (%); B, Fermentation temperature ($^{\circ}$ C); C, Fermentation duration (h); df = Degree of freedom

Table 4: Variance analysis for the fitting of the quadratic model on α -glucosidase inhibitory activity;

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1349.38	9	149.93	93.11	<0.0001	Significant
A	2.56	1	2.56	1.59	0.236	
B	9.28	1	9.28	5.76	0.0373	
C	1.08	1	1.08	0.6726	0.4313	
AB	19.44	1	19.44	12.07	0.006	
AC	12.88	1	12.88	8	0.0179	
BC	0.5253	1	0.5253	0.3262	0.5805	
A^2	232.31	1	232.31	144.28	<0.0001	
B^2	605.99	1	605.99	376.35	<0.0001	
C^2	700.25	1	700.25	434.89	<0.0001	
Residual	16.1	10	1.61			
Lack of Fit	12.29	5	2.46	3.23	0.1121	Not Significant
Pure Error	3.81	5	0.7617			
Cor Total	1365.48	19				
	$R^2 = 0.9882$					

A, Inoculation amount (%); B, Fermentation temperature ($^{\circ}$ C); C, Fermentation duration (h); df = Degree of freedom

The response surface plots in Fig. (3) illustrate the combined effects of these variables. Specifically, Fig. (3A) highlights that the inhibitory effect on α -

glucosidase increased with rising inoculation amount and fermentation temperature, reaching a peak before declining. Figure (3B-C) demonstrates similar trends

with the other parameter combinations, where the inhibition rate generally follows a pattern of increasing to a maximum and then decreasing as the parameters continue to rise. The inhibition rates observed here are substantially higher than those of unfermented samples, suggesting that fermentation significantly boosts the tea's potential for α -glucosidase inhibition.

Determination and Validation of Optimal Fermentation Conditions

Using RSM-CCD, the optimal conditions for maximizing the reduction in the activity of these two enzymes were identified by adjusting the three fermentation parameters. The optimal fermentation parameters for the herbal compound tea, based on the effects of the three independent variables on the suppression of the two enzymes, were determined to be an inoculation amount of 3.88%, a fermentation temperature set at 30.01 °C, and a fermentation duration extending to 73.50 h. To validate the optimized fermentation conditions, triplicate experiments were conducted, confirming that the actual reduction in activity of the two enzymes involved in starch and sugar breakdown closely matched the predicted values (Table 5). The observed inhibition rates observed were 62.21±1.94% for α -amylase and 72.39±1.18% for α -glucosidase, with no statistically significant discrepancies observed between the theoretical and observed outcomes. Furthermore, the inhibitory impact exerted by the fermented compound tea on α -amylase was substantially higher than that exhibited by the unfermented sample at an identical concentration ($p < 0.05$), while the inhibition of α -glucosidase also exhibited a markedly ($p < 0.05$) greater extent in comparison to the unfermented tea.

Comparison of Bioactive Components in Fermented and Unfermented Tea

To evaluate the impact of fermentation on the bioavailability of bioactive components in herbal tea, we

examined changes in key active components, including total phenolics, polysaccharides, flavonoids, proteins, and free amino acids, before and after fermentation. Table (6) illustrates a general elevation in bioactive component content in the fermented tea relative to the unfermented counterpart, indicating an enhancement in bioavailability due to the fermentation process. Specifically, the total phenolic content in fermented tea was 223.16±7.48 GAE mg/g, compared to 185.79±8.51 GAE mg/g in the unfermented tea. Additionally, total polysaccharides in the fermented tea reached 695.48±13.66 μ g/mL, significantly higher than the 572.23±10.29 μ g/mL found in unfermented tea, highlighting fermentation's role in increasing the bioavailability and concentration of polysaccharides.

Evaluation of Antioxidant Activity in Fermented and Unfermented Tea

To comprehensively evaluate the influence of SSF on the oxidative stress-reducing efficacy of the herbal tea, the free radical neutralization abilities against DPPH, ABTS, and hydroxyl radicals were systematically measured and contrasted among the unfermented tea, the fermented tea, and ascorbic acid, which was employed as the positive control. As indicated in Figure (4), the fermented tea consistently exhibited higher scavenging activity across all three radicals compared to unfermented tea. Specifically, the DPPH radical scavenging activity increased from 44.39±1.96% in unfermented tea to 52.71±0.38% in fermented tea. Similarly, the ABTS radical scavenging rate increased from 37.48±1.70% in unfermented tea to 55.30±1.01% in fermented tea. For hydroxyl radicals, the scavenging activity rose from 39.42±0.82% in unfermented tea to 47.39±1.09% in fermented tea. These results suggest that fermentation enhances the free radical neutralization abilities of the herbal tea, a trend observed in similar studies where fermentation led to improved antioxidant activity. Across all assays, ascorbic acid exhibited the highest scavenging rate, consistently surpassing 85%.

Table 5: Predicted and experimental response values at optimum conditions

Response	Predicted value	Experimental value	Unfermented tea	Acarbose (0.05 mg/mL)
α -amylase inhibition rate (%)	63.59	62.21±1.94	43.39±1.35	87.52±2.28
α -glucosidase inhibition rate (%)	72.54	72.39±1.18	46.75±1.27	91.46±2.45

Table 6: Comparison of bioactive component levels in fermented and unfermented tea;

Bioactive components	Fermented tea	Unfermented tea
Total phenolic (GAE mg/g)	223.16±7.48 ^a	185.79±8.51 ^b
Total polysaccharide(μ g/mL)	695.48±13.66 ^a	572.23±10.29 ^b
Total flavonoids (mg/g)	24.12±2.55 ^a	18.48±2.98 ^b
Protein (mg/g)	26.31±1.23 ^a	16.97±2.07 ^b
Free amino acids (mg/g)	34.46±2.82 ^a	22.09±3.26 ^b

The values are expressed as mean \pm standard deviation with $n = 3$. Statistically significant ($p < 0.05$) differences between the fermented and unfermented tea are denoted by different superscript letters (a, b, c)

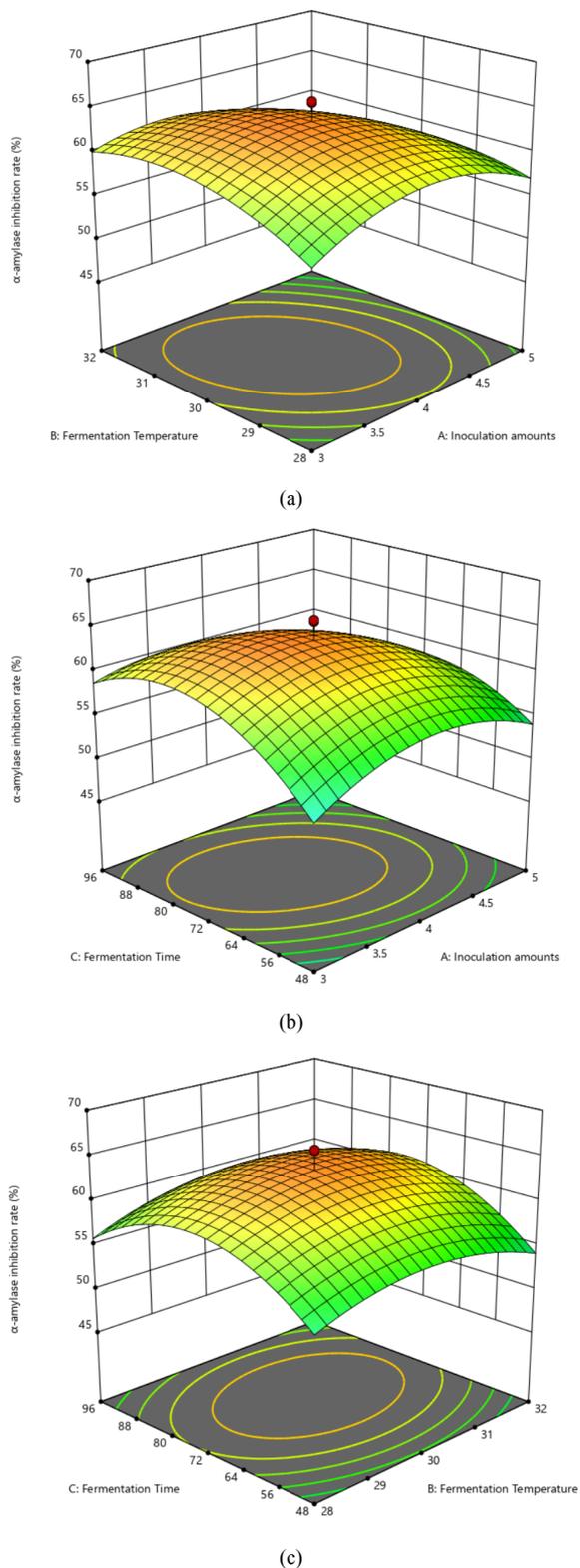


Fig. 2: Three-dimensional response surface plots (3D) illustrating the suppression of α -amylase as influenced by the pivotal interactions between factors. (a) Inoculation volume and fermentation temperature; (b) Inoculation volume and fermentation duration; (c) Fermentation temperature and fermentation duration

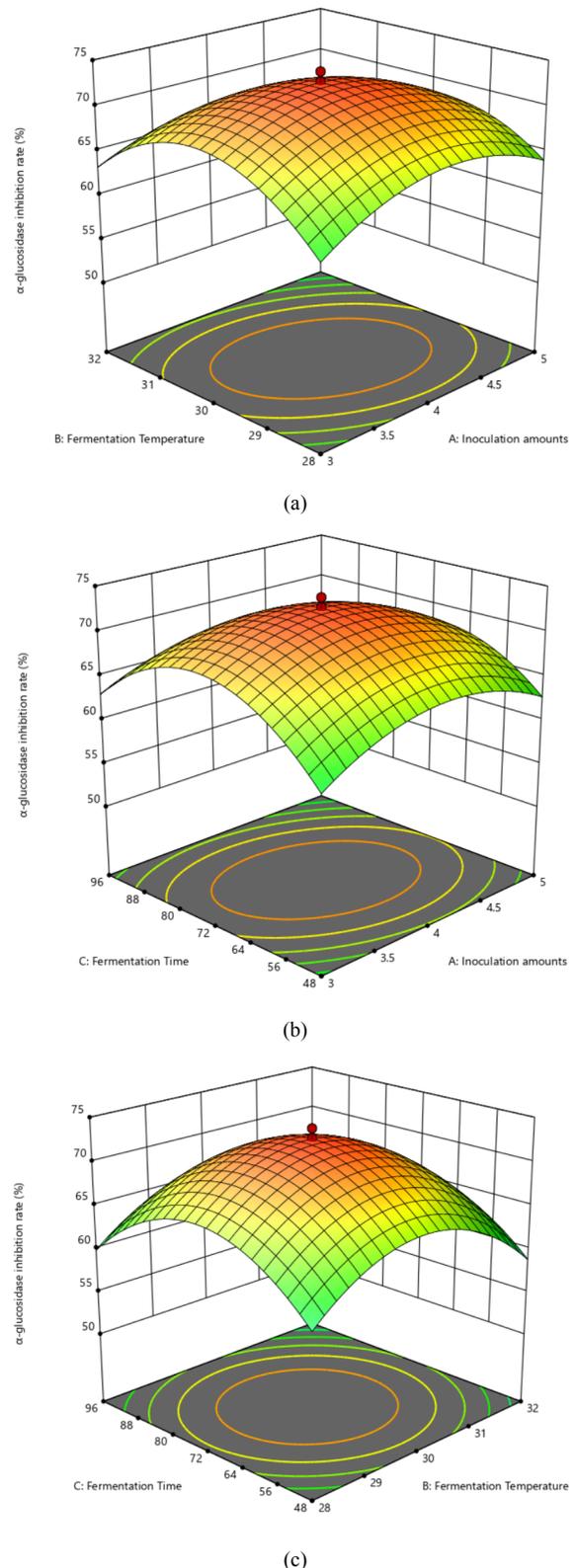


Fig. 3: Three-dimensional response surface plots illustrating the suppression of α -glucosidase as influenced by the pivotal interactions between factors. (a) Inoculation volume and fermentation temperature; (b) Inoculation volume and fermentation duration; (c) Fermentation temperature and fermentation duration

Discussion

Introduction to Hyperglycemia and Natural Products for Blood Glucose Management

In recent years, the prevalence of hyperglycemia has risen dramatically, posing a significant public health challenge. The search for effective, natural alternatives for managing blood glucose levels has gained considerable attention due to the potential for these substances to offer therapeutic benefits with minimal side effects. In this context, the use of natural products, particularly those enriched with bioactive compounds through fermentation, holds promise as an adjunctive therapy for blood glucose regulation. Our study focused on optimizing the fermentation process to enhance the antidiabetic potential of a herbal tea. Through a series of single-factor experiments, key fermentation parameters were identified, which significantly influence the bioactive components of the tea.

Interpretation of Findings and Comparison with Previous Research

Based on the results of the single-factor experiments, the comparison between the fermented and unfermented tea, as well as the positive control (acarbose), demonstrates that fermentation significantly enhances both two enzymes' inhibitory capacities. The fermented tea consistently demonstrated significantly ($p < 0.05$) higher inhibition activity compared to the unfermented (blank) group across different inoculation levels, fermentation temperatures, and fermentation times, with notable increases observed at optimal conditions. Although the fermented tea showed significantly improved ($p < 0.05$) enzyme inhibition compared to the blank group, the positive control (acarbose) exhibited the highest inhibition rates for both enzymes, significantly ($p < 0.05$) outperforming all fermented tea samples.

The results of CCD-RSM indicate that the inhibition of α -amylase by the compound tea infusion is significantly influenced by the inoculation amount, fermentation temperature, and fermentation time, as well as their interactions (Table 3, Figure 2). Specifically, the quadratic effects of these variables suggest a non-linear relationship, where both too low and too high values of inoculation amount, fermentation temperature, and fermentation time can reduce the inhibition effectiveness. This non-linear behavior may be attributed to the complex interplay between enzyme activity and the metabolic products generated throughout the SSF process, including phenolic compounds, flavonoids, and polysaccharides, which can enhance or inhibit α -amylase activity depending on their concentration (Fan *et al.*, 2023). The observed trend of enzyme inhibition activity, which initially increases and then decreases with the rising inoculum level, fermentation time, and

temperature, can be attributed to the dynamic changes in the metabolic activity of *A. niger* KY2295 during fermentation. Initially, as the fermentation parameters increase, the metabolic activity of *A. niger* KY2295 intensifies, leading to the synthesis of diverse secondary metabolites, for instance, organic acids, phenolic compounds, and flavonoids, which contribute to enhanced enzyme inhibition activity. However, as fermentation progresses, the accumulation of these metabolites, along with nutrient depletion and potential pH reduction due to organic acid buildup, may create an unfavorable environment for *A. niger* KY2295. This shift can inhibit further metabolic activity, possibly leading to the degradation or transformation of active compounds, ultimately resulting in a decline in enzyme inhibition activity after reaching its peak (Tao *et al.*, 2022). The interaction effects, particularly between inoculation amount and fermentation time, further underscore the importance of optimizing these parameters to achieve maximum enzyme inhibition.

When comparing these findings with those of Choi *et al.*, it is evident that similar trends were observed in their study (Choi *et al.*, 2022), where the quadratic effects of fermentation parameters also played a significant role. However, unlike the findings of Choi *et al.*, where only the inoculation amount showed a significant interaction effect, our study demonstrates the importance of fermentation time as well. This discrepancy could be due to differences in the microbial strains used or variations in the fermentation medium. Moreover, the significant interaction between inoculation amount and fermentation time observed in our study suggests that careful control of these variables is critical for maximizing the functional properties of the herbal tea extract. This result aligns with previous research regarding the optimization of fermentation processes for bioactive compounds (Li *et al.*, 2023b; Ng *et al.*, 2022; Geraris Kartelias *et al.*, 2023).

Similarly, the inhibition of α -glucosidase was found to be significantly affected by the same fermentation parameters, with bioactive compounds such as polysaccharides and phenolic compounds playing a synergistic role in enhancing the inhibitory activity, with the quadratic terms again playing a critical role. The results highlight that, while inoculation amount and fermentation temperature are key factors, their interaction is particularly important in determining the extent of α -glucosidase inhibition (Table 4, Figure 3). The decrease in inhibition rates observed at higher levels of these parameters suggests that excessive fermentation or high microbial activity might lead to the production of compounds that either counteract the inhibitory effects or degrade the active components responsible for enzyme inhibition (Couto *et al.*, 2017; Hoang *et al.*, 2016).

In contrast to the finding of Hashemi and Jafarpour, where a linear relationship between fermentation time, α -

glucosidase inhibition, and other inflammatory response suppression was reported, our study reveals a more complex interaction, with a non-linear effect being predominant (Hashemi and Jafarpour, 2023). This difference may be attributed to the specific conditions of our fermentation process or the type of compound herbal tea extract used, which differs from the materials used in Hashemi and Jafarpour's research. Additionally, our observation of a significant interaction between inoculation amount and fermentation temperature aligns with the hypothesis that optimal fermentation conditions must balance microbial growth and the stability of bioactive compounds to effectively inhibit α -glucosidase (Nguyen *et al.*, 2017). This nuanced understanding provides a more detailed picture of how these parameters can be fine-tuned to enhance the health-promoting properties of functional foods, a topic that warrants further investigation.

The increased content of bioactive components in fermented tea, particularly phenolic compounds such as chlorogenic acid and quercetin, as well as polysaccharides, plays a crucial role in enhancing its antioxidant capacity. This enhancement is evidenced by the significant rise in total phenolic, polysaccharide, flavonoid, protein, and free amino acid levels (Table 6, Figure 4). These bioactive compounds are directly correlated with the higher DPPH, ABTS, and hydroxyl radical quenching abilities observed in fermented herbal tea. Phenolic compounds, known for their hydrogen-donating abilities, directly contribute to the scavenging of free radicals, further explaining the enhancement of antioxidant performance (Dirar *et al.*, 2019a-b; Vargas-León *et al.*, 2018). Among the phenolic compounds, many bioactive phenolic substances in traditional herbs, such as chlorogenic acid and quercetin in hawthorn, are known for their antioxidant and potential hypoglycemic effects (Kostić *et al.*, 2012). Although these specific compounds were not directly measured in our study, similar compounds may contribute to observed activity enhancements. For instance, studies show that chlorogenic acid, often enhanced by fermentation, exhibits antioxidant and hypoglycemic effects by potentially inhibiting glucose absorption and improving insulin sensitivity (Hoang *et al.*, 2016). Similarly, other phenolic compounds like quercetin can regulate glucose metabolism and reduce insulin resistance (Benabderrahmane *et al.*, 2021; Chen *et al.*, 2016).

In the fermentation process, the breakdown of plant cell walls by *Aspergillus niger* KY2295 is pivotal in the augmentation of bioactive compounds derived from *C. cassia* bark, *G. glabra*, *M. alba* L. leaves, and *C. oxyacantha* L. The microbial enzymes secreted during SSF, such as cellulase, ligninase, and pectinase, effectively degrade cell wall components, thereby increasing the release and bioavailability of bound bioactive compounds. This degradation facilitates the

release of phenolic compounds and polysaccharides, enhancing their antioxidant and hypoglycemic properties. The elevation in polysaccharide content further enhances the hypoglycemic and antioxidant capacity of herbal tea due to their potential role in modulating oxidative stress through various mechanisms, including the stabilization of free radicals (Ganesan and Xu, 2019; Liao *et al.*, 2019). For example, mulberry leaves are rich in active polysaccharides, which have shown blood glucose-lowering and immune-regulating effects (Li *et al.*, 2022). During SSF, the degradation of cell walls may promote the secretion of these polysaccharides, thereby boosting their biological activity (Hu *et al.*, 2024). Additionally, flavonoids are potent antioxidants and contribute significantly to the alleviation of oxidative stress through the elimination of free radicals, which may explain the enhanced inhibition rates of the two enzymes in fermented herbal tea (Long *et al.*, 2022).

The observed increase in protein content in our fermented samples (Table 6) could also be linked to an enhanced antioxidant defense mechanism, as certain proteins and peptides exhibit radical scavenging properties (Jiménez-Ruiz *et al.*, 2013). These findings suggest a direct correlation between bioactive compound enhancement and the tea's antioxidant and hypoglycemic effects (Figure 4). Furthermore, the rise in free amino acids, which possess antioxidant properties, contributes to the overall improvement in the tea's inhibitory capacities on α -amylase and α -glucosidase (Wu *et al.*, 2003).

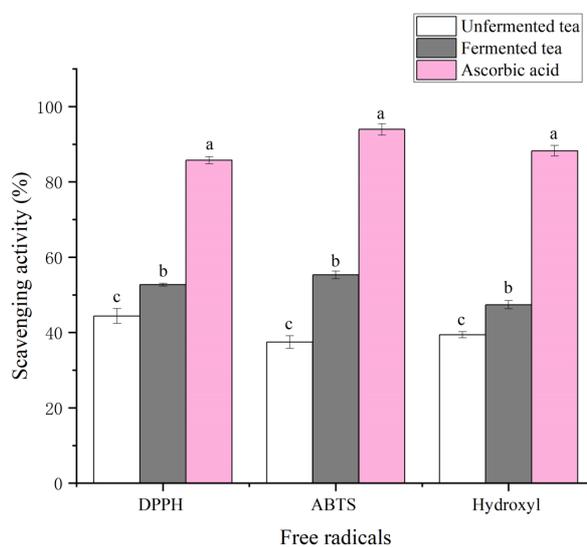


Fig. 4: Scavenging activity of DPPH, ABTS, and Hydroxyl radicals in unfermented tea, fermented tea, and ascorbic acid. The error bars are indicative of the SD obtained from three independent biological replicates. The presence of distinct superscript letters signifies statistically significant disparities between the values ($p < 0.05$)

These improvements in antioxidant capacity, as demonstrated in Figure (4), may be directly associated with the enhanced inhibition of these two enzymes observed in fermented herbal tea, considering the established involvement of oxidative stress in the regulation of these enzymes (Amini *et al.*, 2021). The rise in bioactive components like phenolic compounds and polysaccharides (Table 6) suggests that fermentation enhances both antioxidant and hypoglycemic activities through a synergistic mechanism. By reducing oxidative stress, the bioactive components in fermented tea may help mitigate the damage to pancreatic β -cells, thereby enhancing insulin secretion and glucose metabolism (Gray *et al.*, 2016; Lee *et al.*, 2020). This synergistic effect not only improves hypoglycemic outcomes but also highlights the potential of fermented tea in managing blood glucose levels through multiple mechanisms. Our results align with the findings of Wang and Wang. And Yue *et al.*, where fermentation has been shown to enhance the bioavailability and effectiveness of bioactive compounds, leading to improved health benefits (Yue *et al.*, 2022).

Limitations and Future Research Directions

The results suggest that while fermentation has a significant effect on the enhancement of bioactive components and hypoglycemic activity, further research is warranted to explore variability in fermentation outcomes and to verify these effects *in vivo*. Additionally, exploring the interaction of different microbial strains and their specific metabolic contributions could deepen the understanding of fermentation-induced metabolic changes and expand the application of this process in functional foods.

Conclusion

In this study, *A. niger* KY2295 was employed to perform SSF on a herbal tea composed primarily of *C. cassia* bark, *G. glabra*, *M. abla* L. leaf, and *C. oxyacantha* L., aiming to enhance its auxiliary hypoglycemic efficacy. Through single-factor experiments and CCD-RSM, the optimal fermentation conditions were established. The suppression levels of the digestive enzymes α -amylase and α -glucosidase reached $62.21 \pm 1.94\%$ and $72.39 \pm 1.18\%$, respectively, representing a significant enhancement compared to the unfermented tea. Additionally, the contents of key bioactive components increased notably, including total phenolics (20.11%), flavonoids (30.52%), polysaccharides (21.54%), and free amino acids (55.99%). These increases significantly contributed to the improved hypoglycemic potential and antioxidant capacity. Additionally, DPPH, ABTS, and hydroxyl radical scavenging activities were increased by 18.47, 47.55, and 20.22%, respectively, demonstrating the enhanced antioxidant properties of fermented tea. This research provides a valuable scientific foundation for the development of natural, effective, and side-effect-free functional herbal teas with hypoglycemic properties,

offering new perspectives for advancing the functional food industry. Future research should focus on elucidating the specific metabolic pathways activated during SSF and confirming the *in vivo* hypoglycemic effects.

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Author's Contributions

Qing Liu: Contributed to the project conceptualization, experimental methods, and manuscript drafting.

Xiaowen Que, Yueping Yang and Jinghao Zhao: Participated in the fermentation process, indicators determination, and data curation.

Tian Tian, Jiabin Wang and Zhaoshen Fan: Participated in the formulation consultation.

Hua Fang: Kindly provide the stains.

Yuanda Song: Participate in experiment guidance and manuscript revision.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

References

- Dujnič, V., Matulová, M., Chyba, A., & Pätoprstý, V. (2021). Polysaccharides in *Siraitia Grosvenori* Flowers and Herbal Tea. *Chemical Papers*, 75(3), 1175-1185.
<https://doi.org/10.1007/s11696-020-01347-3>
- Fan, Z., Chen, T., Cai, G., Huang, X., Zhong, S., Li, X., & Zhang, E. (2023). Effect of *Aspergillus Niger* Fermentation on the Metabolites in Corn Stalks. *Fermentation*, 9(1), 50.
<https://doi.org/10.3390/fermentation9010050>

- Ganesan, K., & Xu, B. (2019). Antidiabetic Effects and Mechanisms of Dietary Polysaccharides. *Molecules*, 24(14), 2556.
<https://doi.org/0.3390/molecules24142556>
- Geraris Kartelias, I., Karantonis, H. C., Giaouris, E., Panagiotakopoulos, I., & Nasopoulou, C. (2023). Kombucha Fermentation of Olympus Mountain Tea (*Sideritis Scardica*) Sweetened with Thyme Honey: Physicochemical Analysis and Evaluation of Functional Properties. *Foods*, 12(18), 3496.
<https://doi.org/10.3390/foods12183496>
- Gnoumou, E., Kongpichitchoke, T., & Jose, A. (2023). Effect of Solid-State Fermentation (SSF) on the Antioxidant and Hypoglycemic Activities of Jackfruit (*Artocarpus Heterophyllus* Lam.) By-Products using *Aspergillus Niger*, *Aspergillus Oryzae* and *Rhizopus Oryzae*. *Trends in Sciences*, 20(10), 5648.
<https://doi.org/10.48048/tis.2023.5648>
- Gray, J. P., Zayasbaban Burgos, D., Yuan, T., Seeram, N., Rebar, R., Follmer, R., & Heart, E. A. (2016). Thymoquinone, a Bioactive Component of *Nigella Sativa*, Normalizes Insulin Secretion from Pancreatic β -Cells Under Glucose Overload Via Regulation of Malonyl-Coa. *American Journal of Physiology-Endocrinology and Metabolism*, 310(6), E394-E404.
<https://doi.org/10.1152/ajpendo.00250.2015>
- Hashemi, S. M. B., & Jafarpour, D. (2023). Lactic Acid Fermentation of Guava Juice: Evaluation of Nutritional and Bioactive Properties, Enzyme (α -Amylase, α -Glucosidase, and Angiotensin-Converting Enzyme) Inhibition Abilities, and Anti-Inflammatory Activities. *Food Science and Nutrition*, 11(12), 7638-7648.
<https://doi.org/10.1002/fsn3.3683>
- Hoang, N. X., Ferng, S., Ting, C.-H., Huang, W.-H., Chiou, R. Y.-Y., & Hsu, C.-K. (2016). Optimizing the Initial Moromi Fermentation Conditions to Improve the Quality of Soy Sauce. *LWT*, 74, 242-250. <https://doi.org/10.1016/j.lwt.2016.07.049>
- Hu, Y., Zhang, Y., Cui, X., Wang, D., Hu, Y., & Wang, C. (2024). Structure-Function Relationship and Biological Activity of Polysaccharides from Mulberry Leaves: A Review. *International Journal of Biological Macromolecules*, 268, 131701.
<https://doi.org/10.1016/j.ijbiomac.2024.131701>
- Huang, Q., Zhang, H., & Xue, D. (2019). Enhancement of the Antioxidant and Hypolipidemic Activities of *Puerariae Radix* by Fermentation with *Aspergillus Niger*. *Food Science and Biotechnology*, 28(4), 1117-1124.
<https://doi.org/10.1007/s10068-018-0540-9>
- Janarny, G., & Gunathilake, K. D. P. P. (2020). Changes in Rice Bran Bioactives, their Bioactivity, Bioaccessibility and Bioavailability with Solid-State Fermentation by *Rhizopus Oryzae*. *Biocatalysis and Agricultural Biotechnology*, 23, 101510.
<https://doi.org/10.1016/j.bcab.2020.101510>
- Jiménez-Ruiz, E. I., Calderón de la Barca, A. M., Sotelo-Mundo, R. R., Arteaga-Mackinney, G. E., Valenzuela-Melendez, M., & Peña-Ramos, E. A. (2013). Partial Characterization of Ultrafiltrated Soy Protein Hydrolysates with Antioxidant and Free Radical Scavenging Activities. *Journal of Food Science*, 78(8), C1152-C1158.
<https://doi.org/10.1111/1750-3841.12200>
- Joshua, V. A., Sanusi, M. S., Abiodun, O. A., Kayode, B. I., Olabanji, S. O., Egwumah, O. R., & Kayode, R. M.-O. (2023). Quality and Sensory Attributes of Composite Herbal Tea from *Parquetina Nigrescens* (*Parquetina*) and *Cymbopogon Citratus* (*Lemongrass*). *Journal of Food Measurement and Characterization*, 17(3), 2730-2740.
<https://doi.org/10.1007/s11694-023-01830-x>
- Kostić, D. A., Velicković, J. M., Mitić, S. S., Mitić, M. N., & Randelović, S. S. (2012). Phenolic Content and Antioxidant and Antimicrobial Activities of *Crataegus Oxyacantha* L (Rosaceae) Fruit Extract from Southeast Serbia. *Tropical Journal of Pharmaceutical Research*, 11(1), 117-124.
<https://doi.org/10.4314/tjpr.v11i1.15>
- Lee, D., Lee, J. S., Sezirahiga, J., Kwon, H. C., Jang, D. S., & Kang, K. S. (2020). Bioactive Phytochemicals Isolated from *Akebia quinata* Enhances Glucose-Stimulated Insulin Secretion by Inducing PDX-1. *Plants*, 9(9), 1087.
<https://doi.org/10.3390/plants9091087>
- Li, J., Ji, T., Su, S., Zhu, Y., Chen, X., Shang, E., Guo, S., Qian, D., & Duan, J. (2022). Mulberry Leaves Ameliorate Diabetes Via Regulating Metabolic Profiling and *Ages/Rage* and *P38 Mapk/Nf-kb* Pathway. *Journal of Ethnopharmacology*, 283, 114713. <https://doi.org/10.1016/j.jep.2021.114713>
- Li, P., Tan, J., Xiao, M., Cai, X., Xue, H., & Yu, H. (2023). Bioactive Substances and Biological Functions in *Malus Hupehensis*: A Review. *Molecules*, 28(2), 658.
<https://doi.org/10.3390/molecules28020658>
- Li, Q., Liu, L., Zhang, J., Dong, M., Wang, L., Julian McClements, D., Fu, Y., Han, L., Shen, P., & Chen, X. (2023). Effects of Pile Fermentation on the Physicochemical, Functional, and Biological Properties of Tea Polysaccharides. *Food Chemistry*, 410, 135353.
<https://doi.org/10.1016/j.foodchem.2022.135353>
- Liao, Z., Zhang, J., Liu, B., Yan, T., Xu, F., Xiao, F., Wu, B., Bi, K., & Jia, Y. (2019). Polysaccharide from Okra (*Abelmoschus esculentus* (L.) Moench) Improves Antioxidant Capacity via *PI3K/AKT* Pathways and *Nrf2* Translocation in a Type 2 Diabetes Model. *Molecules*, 24(10), 1906.
<https://doi.org/10.3390/molecules24101906>
- Liu, N., Wang, Y., An, X., & Qi, J. (2022). Study on the Enhancement of Antioxidant Properties of Rice Bran Using Mixed-Bacteria Solid-State Fermentation. *Fermentation*, 8(5), 212.
<https://doi.org/10.3390/fermentation8050212>

- Liu, Q., Shi, W., Xue, F., Wu, C., Mohamed, H., Li, M., & Song, Y. (2022). Phytochemical Profiles and Antidiabetic Potential of the Extract of *Pueraria lobata* Compound Teabags: Preparation and Optimization Infusion Conditions. *American Journal of Biochemistry and Biotechnology*, 18(4), 405-416.
<https://doi.org/10.3844/ajbbsp.2022.405.416>
- Liu, Q., Wu, H., Wu, Y., Liu, M., Zhang, Y., & Song, Y. (2022). Evaluation of Antioxidant and Antibacterial Activities of the Polysaccharides from Mycelium and the Culture Medium of *Pleurotus Eryngii* Strain UN-1. *American Journal of Biochemistry and Biotechnology*, 18(3), 261-270.
<https://doi.org/10.3844/ajbbsp.2022.261.270>
- Long, H.-P., Liu, J., Xu, P.-S., Xu, K.-P., Li, J., & Tan, G.-S. (2022). Hypoglycemic Flavonoids from *Selaginella Tamariscina* (P.Beauv.) Spring. *Phytochemistry*, 195, 113073.
<https://doi.org/10.1016/j.phytochem.2021.113073>
- Ma, W., Xiao, L., Liu, H., & Hao, X. (2022). Hypoglycemic Natural Products With in Vivo Activities and Their Mechanisms: A Review. *Food Science and Human Wellness*, 11(5), 1087-1100.
<https://doi.org/10.1016/j.fshw.2022.04.001>
- Malongane, F., McGaw, L. J., Debusho, L. K., & Mudau, F. N. (2020). Sensory Characteristics and Volatile Compounds of Herbal Teas and Mixtures of Bush Tea with Other Selected Herbal Teas of South Africa. *Foods*, 9(4), 496.
<https://doi.org/10.3390/foods9040496>
- Ng, Z. X., Soh, E. Y. W., & Yong, P. H. (2022). The Influence of Fermentation and Drying Methods on the Functional Activities and Sensory Quality of *Artemisia Argyi* H.Lév. Vaniot Herbal Tea. *Journal of Applied Research on Medicinal and Aromatic Plants*, 30, 100393.
<https://doi.org/10.1016/j.jarmap.2022.100393>
- Nguyen, V., Nguyen, A., & Wang, S.-L. (2017). Utilization of Fishery Processing By-Product Squid Pens for α -Glucosidase Inhibitors Production by *Paenibacillus* sp. *Marine Drugs*, 15(9), 274.
<https://doi.org/10.3390/md15090274>
- Özcan, M. M., & Matthäus, B. (2017). A Review: Benefit and Bioactive Properties of Olive (*Olea Europaea* L.) Leaves. *European Food Research and Technology*, 243(1), 89-99.
<https://doi.org/10.1007/s00217-016-2726-9>
- Pantidos, N., Boath, A., Lund, V., Conner, S., & McDougall, G. J. (2014). Phenolic-Rich Extracts from the Edible Seaweed, *Ascophyllum Nodosum*, Inhibit α -Amylase and α -Glucosidase: Potential Anti-Hyperglycemic Effects. *Journal of Functional Foods*, 10, 201-209.
<https://doi.org/10.1016/j.jff.2014.06.018>
- Tao, A., Feng, X., Sheng, Y., & Song, Z. (2022). Optimization of the *Artemisia* Polysaccharide Fermentation Process by *Aspergillus niger*. *Frontiers in Nutrition*, 9.
<https://doi.org/10.3389/fnut.2022.842766>
- Vargas-León, E. A., Díaz-Batalla, L., González-Cruz, L., Bernardino-Nicanor, A., Castro-Rosas, J., Reynoso-Camacho, R., & Gómez-Aldapa, C. A. (2018). Effects of Acid Hydrolysis on the Free Radical Scavenging Capacity and Inhibitory Activity of the Angiotensin Converting Enzyme of Phenolic Compounds of Two Varieties of Jamaica (*Hibiscus Sabdariffa*). *Industrial Crops and Products*, 116, 201-208.
<https://doi.org/10.1016/j.indcrop.2018.02.044>
- Wang, X., & Wang, P. (2023). Red Beetroot Juice Fermented by Water Kefir Grains: Physicochemical, Antioxidant Profile and Anticancer Activity. *European Food Research and Technology*, 249(4), 939-950.
<https://doi.org/10.1007/s00217-022-04185-7>
- Wen, F., Li, P., Zhang, Y., Zhong, H., Yan, H., & Su, W. (2023). Preparation, Characterization of Green Tea Carbon Quantum Dots/Curcumin Antioxidant and Antibacterial Nanocomposites. *Journal of Molecular Structure*, 1273, 134247.
<https://doi.org/10.1016/j.molstruc.2022.134247>
- Wu, H.-C., Shiau, C.-Y., Chen, H.-M., & Chiou, T.-K. (2003). Antioxidant Activities of Carnosine, Anserine, Some Free Amino Acids and Their Combination. *Journal of Food and Drug Analysis*, 11(2). <https://doi.org/10.38212/2224-6614.2720>
- Xue, H., Hao, Z., Gao, Y., Cai, X., Tang, J., Liao, X., & Tan, J. (2023). Research Progress on the Hypoglycemic Activity and Mechanisms of Natural Polysaccharides. *International Journal of Biological Macromolecules*, 252, 126199.
<https://doi.org/10.1016/j.ijbiomac.2023.126199>
- Yue, Q., Wang, Z., Yu, F., Tang, X., Su, L., Zhang, S., Sun, X., Li, K., Zhao, C., & Zhao, L. (2022). Changes In Metabolite Profiles and Antioxidant and Hypoglycemic Activities of *Laminaria Japonica* After Fermentation. *LWT*, 158, 113122.
<https://doi.org/10.1016/j.lwt.2022.113122>
- Zhang, L., Qiao, H.-Y., Liu, H.-X., Jiang, G.-C., Wang, L.-Y., & Liu, X.-J. (2022). Antioxidant, Hypoglycemic and Protection of Acute Liver Injury Activities of *Ganoderma Lucidum* Spore Water Extract. *Journal of Functional Foods*, 97, 105254.
<https://doi.org/10.1016/j.jff.2022.105254>
- Zhang, X., Zhang, L., Zhang, B., Liu, K., Sun, J., Li, Q., & Zhao, L. (2022). Herbal Tea, a Novel Adjuvant Therapy for Treating Type 2 Diabetes Mellitus: A Review. *Frontiers in Pharmacology*, 13.
<https://doi.org/10.3389/fphar.2022.982387>