

Research Article

Enrichment of Bioactive Compounds from Lotus (*Nelumbo nucifera* 'Roseum Plenum') Tea through Fermentation

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ABSTRACT

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Lotus tea, tea made from the Double red lotus (*Nelumbo nucifera* 'Roseum Plenum'). This advantageous characteristic is caused by its bioactive compounds, which include flavonoids and alkaloids. However, stirring the tea with hot water rapidly extracts the active compounds, which may result in a lower overall yield. Additionally, the related flavonoids and alkaloids have good solubility in alcohol. This study aimed to evaluate the feasibility of using lotus tea as a substrate for wine fermentation and to investigate the effect of fermentation on the extraction and enrichment of bioactive compound. Lotus tea and tea infusion used as substrate for the fermentation and HPLC utilized for measuring interesting bioactive compounds. The results indicate that both lotus tea and tea infusion underwent steady fermentation, producing ethanol levels of approximately 6% (v/v) within the first 7 days. All samples displayed characteristics typical of sweet wine, with alcohol content ranging from 6-8% (v/v) by day 28. Fermentation increased the concentrations of bioactive compounds, including catechin, epigallocatechin gallate (EGCG), and nuciferine, to approximately 2-5 times those in the original tea infusion, indicating enhanced extraction during fermentation. While fermentation increased the content of bioactive compounds in lotus tea, further in vivo and clinical investigations are necessary to establish the biological relevance of these findings and assess any potential health effects.

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1. Introduction

The lotus flower (*Nelumbo nucifera* Gaertn) is a plant commonly used in the daily lives of Thai Buddhists and has also been used in traditional medicine. Especially the Sattabongkot cultivar, double red lotus (*Nelumbo nucifera* "Roseum Plenum"), claimed to support neurological function, cardiovascular health, antioxidant, reduce inflammation, improve sleep quality, and antidiabetic activities (Mukherjee *et al.*, 2009; Tungmunthum *et al.*, 2018). The bioactive flavonoids and alkaloids present in lotus tissues are the main reasons for these health benefits.

Lotus tea made from lotus flower, stamen and petal and aromatic herbs. The main bioactive compounds as effect to health like quercetin, kaempferol, catechin, and nuciferine were limited water solubility (Lin *et al.*, 2009; Sharma *et al.*, 2017). Additionally, hot- water infusion is the common method for

preparing lotus tea; however, this approach leads to inefficient extraction, resulting in beverages with suboptimal phytochemical content and diminished health benefits (Chen *et al.*, 2012).

Ethanol is widely used as an effective solvent for extracting flavonoids and alkaloids. However, the high alcohol concentration beverage is unsuitable for functional foods and health products because of safety, sensory, and regulatory concerns. Wine, alcoholic fermentation is the strategy that combines the advantage in food processing with a healthy perspective. Ethanol produced during the fermentation process acts as a green extraction solvent, facilitating the extraction of bioactive compounds from plant materials (Chemat *et al.*, 2012). During alcoholic fermentation, ethanol concentrations typically remain below 14% (v/v), enabling the gradual extraction of compounds with limited water solubility. In addition, ethanol generated by yeast enhances the solubility and extraction

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efficiency of relatively less polar phytochemicals, while microbial enzymes degrade plant cell walls, promoting the release of intracellular bioactive constituents. Fermentation also induces biochemical transformations and structural modifications within the plant matrix, which can improve the release, stability, and bioaccessibility of phytochemicals, including flavonoids and alkaloids (Hussain *et al.*, 2016; Luo *et al.*, 2024). Collectively, these processes contribute to the enrichment of bioactive compounds in fermented lotus tea products.

The aim of this study was to utilize alcoholic wine fermentation as a method to enhance the extraction of bioactive flavonoids and alkaloids from lotus tea. We compared the phytochemical profiles of lotus tea infusion and fermented lotus wine, with a focus on weakly water-soluble compounds such as kaempferol and nuciferine. We hypothesized that the gradual increase in low-level ethanol concentration (approximately 6–8% v/v) over 28 days of fermentation would improve the solubilization of these compounds. This process would result in a fermented lotus tea beverage with higher bioactive content.

2. Materials and Methods

2.1 Raw materials and yeast culture

Dried lotus tea, stamens, petals, and herbal ingredients as aroma herb were sourced from the community enterprise Thanacha Wellness Organic Farm (Chonburi, Thailand). The commercial active dry yeast, *Saccharomyces cerevisiae* from Lalvin EC-1118™ (Lallemand Inc, Montreal, Canada).

2.2 Preparation of lotus tea wine

Lotus tea wine fermentation was performed using tea powder, lotus stamens, and petals in four treatments. The quantities of lotus stamens (24 g) and petals (96 g) were equivalent to their respective proportions in 150 g of the lotus tea mixture (Table 1).

Table 1 Raw materials and ingredient composition of the wine.

Treatment	Sample	Raw materials	Weight (g)
1	Tea infusion	Tea powder	150
2	Tea	Tea powder	150
3	Stamen	Lotus stamen	24
4	Petals	Lotus petals	96

Nine liters of water were prepared, and granulated sugar was added to adjust the total soluble solids (TSS) to 20 °Brix, as sugar composition can influence yeast metabolism. Ingredients were added according to each treatment and boiled for 30 minutes. Solid residues were removed, leaving approximately 20%, except in the tea treatment (treatment 2) where residues were retained. The mixture was cooled and transferred to a 10 L fermentation tank.

Prior to inoculation, 5 g of active dry yeast (Lalvin EC-1118™, Canada) was added to 100 mL of warm lotus tea infusion and incubated for 30 min to obtain a rehydrated starter

culture. Fermentation was conducted at an inoculation rate of 0.5 g/L and carried out at ambient temperature (28–30 °C) for 28 days. After fermentation, the clear supernatant was collected, bottled, and stored at 4 °C.

2.3 Monitoring of the fermentation process

Samples of fermentation were collected every seven days. A hand refractometer was used to measure TSS as °Brix using the AOAC method (AOAC, 2019). The pH was determined using a pH meter (pH 700, Eutech Instruments, Singapore) following the manufacturer's recommended. Ebulliometry was used to determine the alcohol content in accordance with OIV-MA-AS312-01A (OIV, 2012). Results are shown as mean ± standard deviation (SD), and all experiments were conducted in triplicate.

2.4 Sample and standard solution preparation for bioactive compound analysis

The lotus tea wine sample underwent direct analysis. For the tea infusion samples, one tea bag (containing 2 g of tea powder) was steeped in 250 mL of hot water for five minutes to prepare the first tea infusion samples. To prepare ethanol extracts, petals (9.6 g) were soaked in 10 mL of 70% (v/v) ethanol as a conventional solvent system and extracted at ambient temperature (28–30 °C) for 28 days.

The HPLC reference standards, quercetin hydrate, kaempferol hydrate, catechin hydrate, nuciferine, and troxerutin were obtained from TCI (Tokyo, Japan) and epigallocatechin gallate (EGCG) from Sigma-Aldrich (St. Louis, MO, USA). Stock standard solutions (100 ppm) were prepared in a mobile phase consisting of 1% (v/v) acetic acid in deionized water and acetonitrile (9:1, v/v). All samples and standards solutions were filtered through a 0.22 µm nylon syringe filter prior to HPLC analysis.

2.5 HPLC analysis of bioactive compounds

Bioactive compounds were analyzed using a Shimadzu Prominence-i LC-2050C i-Series HPLC system equipped with a UV-Vis detector (Shimadzu Corporation, Tokyo, Japan). Separation was performed on a reverse-phase C18 column (Shim-pack GIST, 4.6 × 250 mm, 3 µm particle size). The mobile phase consisted of solvent A (1% acetic acid in water) and solvent B (acetonitrile) under isocratic elution (90:10, v/v). The flow rate was 0.7 mL/min, column temperature was maintained at 35 °C, injection volume was 10 µL, and total run time was 60 min. Detection was carried out at 272 nm.

Compound identification was based on retention time comparisons with authentic standards analyzed under identical conditions. Quantification was achieved using external calibration curves derived from peak areas, following the method described by Seal (Seal, 2016).

2.6 Statistical analysis

All fermentation treatments were conducted in triplicate. Statistical analyses were performed using GraphPad Prism

version 9 (GraphPad Software, San Diego, CA, USA). Data for total soluble solids (TSS), alcohol content, and selected bioactive compound concentrations were analyzed by one-way analysis of variance (ANOVA). When significant differences were detected, means were compared using Tukey's honestly significant difference (HSD) post hoc test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1 Feasibility of lotus tea as a substrate for wine fermentation

The use of non-fruit plant or herbs as fermentation substrates could markedly reduce yeast growth and fermentation efficiency. Because of variations in nutrient composition and the presence of inhibitory phytochemicals (Fletcher and Baetz, 2020). These results have demonstrated the potential of lotus tea as a raw material for wine fermentation. The level of alcohol and alterations in TSS serve as the primary criterion for wine characterization to identify wine fermentation performance.

The initial sugar concentration of 20 °Brix (~200 g/L) theoretically support a maximum alcohol yield of approximately 11–12% (v/v) (Duncan and Acton, 1967). In this study, alcohol production of tea infusion, tea and petals treatment at day 7 reached approximately 6–7% (v/v), representing about 50–70% of the theoretical yield, suggesting that fermentation had progressed into the late fermentation phase (Figure 1-2, Table 2).

The absence of significant differences ($p > 0.05$) in both TSS reduction and alcohol production between days 7, 14, and 28 for tea infusion, tea and petals treatment indicates that fermentation may have effectively ceased after 7 days. This behavior is consistent with typical yeast fermentation kinetics, where rapid sugar conversion occurs within the first few days, followed by a plateau phase.

In contrast, lotus stamens showed continued changes up to day 14, implying prolonged fermentation. This may be attributed to its lower nutrient availability or the presence of bioactive compounds that inhibit yeast activity, delaying alcohol production. This fermentation process of lotus tea sample is reliable with previous reports on non-fruit and herbal wine production, which often requires extended fermentation periods depending on its raw material composition (Awasthi, 2022; Shaik *et al.*, 2023).

On day 28 of fermentation, all lotus tea wine exhibited an alcohol content of 6–8% (v/v) and TSS of 7–11 °Brix, with a clear brown appearance (Figure 2). Based on alcohol and TSS levels, the product can be classified as a sweet white wine (Jackson, 2020).

To further confirm the type and concentration of sugars present, the addition HPLC analysis was conducted on both lotus tea infusion and tea wine samples. The results revealed that fructose was the predominant sugar in both types of wine, with concentrations of 1.13 g/100 mL and 0.83 g/100 mL,

respectively. This is consistent with yeast metabolism, where glucose is preferentially utilized over fructose during fermentation (Fleet, 1990).

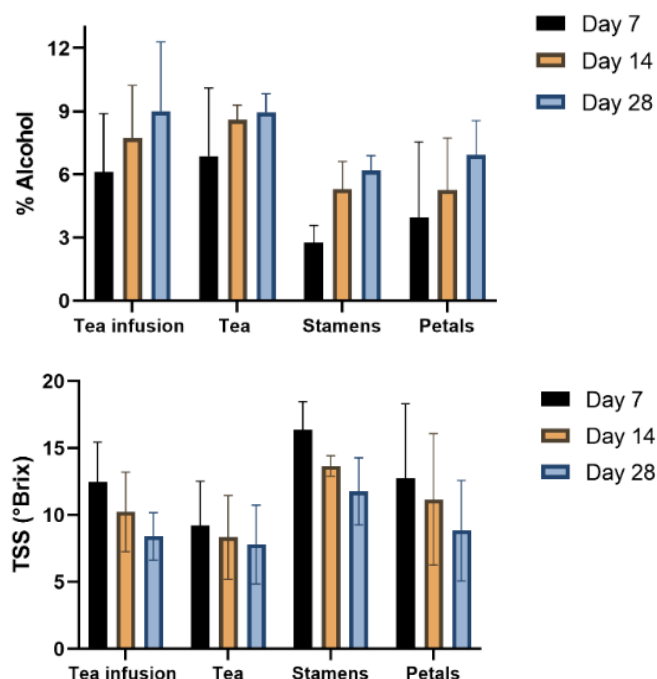


Figure 1 Alcohol content (up) and TSS (low) of lotus tea samples during wine fermentation.



Figure 2 Color of lotus wine after 28 days of fermentation (from left to right): Petals wine, stamens wine, tea infusion wine, and lotus tea wine.

3.2 Effect of wine fermentation on the extraction of bioactive compounds

The extraction efficiency of bioactive compounds from lotus tea, tea infusion, stamens, and petals using a wine fermentation process combined with maceration for 28 days was evaluated and compared with conventional hot water extraction. The total concentration of bioactive compounds varied significantly based on the solvent properties and extraction conditions, as demonstrated by quantitative analysis using HPLC. (Figure 3 and Table 3).

Flavonoids, including quercetin, kaempferol, catechin, and EGCG, as well as alkaloids such as nuciferine, exhibited distinct solubility behaviors in aqueous and alcoholic media, which

directly influenced their extraction efficiency (Dai and Mumper, 2010; Nguyen *et al.*, 2021).

EGCG, which is highly soluble in both water and organic solvents (Liu *et al.*, 2019), was detected at approximately two-fold higher levels in tea wine than in hot water extracts. No significant difference was observed between tea infusion wine and tea wine, indicating that residual tea material during fermentation did not affect extraction yield. The detection of EGCG in stamens and petals wines further confirms its origin from the lotus plant.

Catechin, which has limited solubility in water but increased solubility at higher temperatures and in alcohol (Cuevas-Valenzuela *et al.*, 2014), was detected at higher levels in tea wine than in tea infusion wine. This result suggests that ethanol enhances the extraction of moderately polar compounds, while lotus tea material during fermentation further contributes to increased extraction yield.

Quercetin exhibits low water solubility, with increased solubility under alkaline conditions and precipitation in acidic environments (pH < 4) (Srinivas *et al.*, 2010). Accordingly, only trace amounts were detected in tea infusion and lotus tea wines and none in stamen and petal wines, likely due to the acidic conditions (pH 3–4) after 28 days of fermentation. In contrast, ethanol extracts of lotus petals, which does not create acidic conditions, yielded higher quercetin levels.

Kaempferol, a flavonoid with strong antioxidant activity but very low water solubility (Deng *et al.*, 2019), was detected in minimal amounts in aqueous-based extracts and was absent in systems with higher water content. The highest levels were observed in lotus petals ethanol extracts, consistent with its preference for less polar solvents.

Nuciferine, an alkaloid with negligible water solubility but high solubility in organic solvents such as ethanol, methanol, and chloroform (Du *et al.*, 2016), was found in significantly higher concentrations in tea wine and was approximately four times higher than in tea infusions. The highest levels were detected in petals ethanol extracts, reflecting both solvent compatibility and compound distribution within plant tissues.

This study demonstrates that integrating wine fermentation with maceration is an effective green extraction strategy for enhancing the recovery of bioactive compounds, particularly those with high alcohol solubility. However, solvent composition, pH, and the solubility characteristics of individual compounds remain critical factors for maximizing the concentration of bioactive compounds in the extract.

3.3 Functional implications

The presence of interested flavonoids and alkaloids, including catechin, EGCG, quercetin and nuciferine, in lotus tea wine highlights its potential as a functional beverage with multiple health-promoting properties. These compounds have been extensively reported to exhibit antioxidant, anti-inflammatory, immunomodulatory, and cardioprotective effects

(Chen *et al.*, 2019; Zhao *et al.*, 2023). The enrichment of such bioactive constituents through fermentation, with levels increasing approximately more than twofold compared to the original tea infusion, suggests that the fermentation process may enhance the extraction, transformation, or stability of these compounds.

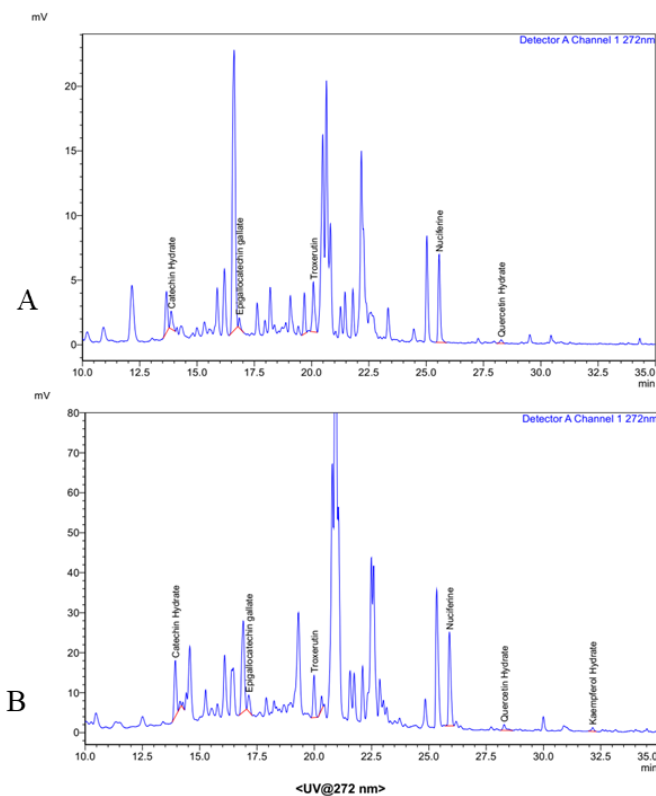


Figure 3 HPLC chromatogram of selected bioactive compounds in tea infusion (A) and lotus tea wine (B).

In particular, nuciferine has attracted attention due to its potential neuropharmacological effects. Previous studies have demonstrated that nuciferine may improve sleep quality by modulating multiple neurotransmitter systems and reducing neuronal hyperexcitability associated with insomnia (Zhang *et al.*, 2024). These findings highlight the potential of lotus tea wine as a functional beverage, with benefits extending beyond its increased nuciferine concentration to potential neuroprotective and other health-promoting effects.

However, it is important to note that the actual health benefits derived from these compounds are influenced not only by their increased concentrations but also by factors such as daily intake, bioavailability, metabolism, and individual physiological variability. Polyphenols such as catechin and EGCG are known to have limited bioavailability due to rapid metabolism and low absorption rates, which may reduce them in vivo efficacy (Cai *et al.*, 2018; Yang *et al.*, 2025).

4. Conclusion

This study demonstrates that lotus tea is a feasible alternative substrate for wine fermentation, despite the inherent

limitations of non-fruit raw materials. The fermentation process successfully supported yeast activity and moderate ethanol production of approximately 6–8% (v/v), which corresponds to about 50–70% of the theoretical yield based on an initial sugar concentration. In addition to ethanol production, Lotus tea wine markedly increased the concentrations of several bioactive constituents, particularly catechin, epigallocatechin gallate

(EGCG), and the poorly water-soluble alkaloid nuciferine, which were present at levels approximately 2–5-fold higher than those in the corresponding tea infusions. These findings demonstrate that lotus tea wine fermentation not only enables ethanol production but also improves the recovery of bioactive phytochemicals, especially compounds with limited water solubility.

Table 2 Changes in TSS (°Brix) and alcohol concentration (% v/v) during wine fermentation.

No.	Treatment	Day 7		Day 14		Day 28	
		TSS (°Brix)	Alcohol (% v/v)	TSS (°Brix)	Alcohol (% v/v)	TSS (°Brix)	Alcohol (% v/v)
1	Tea infusion	12.46 ± 2.96 ^a	6.09 ± 2.79 ^a	10.23 ± 2.97 ^a	7.74 ± 2.48 ^a	8.40 ± 1.76 ^a	8.99 ± 3.29 ^a
2	Tea	9.20 ± 3.32 ^{ab}	6.87 ± 3.22 ^a	8.33 ± 3.12 ^{ab}	8.59 ± 0.69 ^{ab}	7.80 ± 2.94 ^a	8.95 ± 0.87 ^{ab}
3	Stamens	16.36 ± 2.10 ^{ac}	2.77 ± 0.81 ^a	13.66 ± 0.75 ^{ac}	5.30 ± 1.31 ^{ac}	11.76 ± 2.50 ^a	6.19 ± 0.70 ^{ac}
4	Petals	12.73 ± 5.57 ^{abc}	3.96 ± 3.58 ^a	11.16 ± 4.90 ^{abc}	5.25 ± 2.47 ^{abc}	8.83 ± 3.75 ^a	6.93 ± 1.62 ^{abc}

*Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences (p < 0.05)

Table 3 Concentrations of selected bioactive compounds in tea infusion, tea wine and ethanol extracts (mg/mL).

Sample	EGCG	Catechin	Quercetin	Kaempferol	Nuciferine
Tea infusion	1.18 ± 0.13 ^a	2.37 ± 1.66 ^a	0.29 ± 0.00 ^a	0.06 ± 0.00 ^a	1.84 ± 1.30 ^a
Tea infusion wine	2.85 ± 0.26 ^b	1.27 ± 0.51 ^{ab}	0.27 ± 0.01 ^{ab}	0.32 ± 0.07 ^{ab}	4.10 ± 0.69 ^b
Tea wine	2.67 ± 0.38 ^b	11.70 ± 0.87 ^{ac}	0.49 ± 0.15 ^{ac}	0.10 ± 0.02 ^{ac}	5.13 ± 1.98 ^b
Stamens wine	1.21 ± 0.01	1.15 ± 0.51	ND	0.06 ± 0.00	0.68 ± 0.11
Petals wine	2.48 ± 0.27	0.71 ± 0.27	ND	0.06 ± 0.00	4.18 ± 0.20
Petals ethanol extract	15.97 ± 00.30	11.87 ± 0.45	1.06 ± 1.10	2.53 ± 0.03	46.41 ± 0.79

*Data are expressed as mean ± SD (n = 3 for tea infusion, stamens and petals wine and petals ethanol extract; n = 7 for tea and tea infusion wine). Different superscript letters within the same column indicate significant differences (p ≤ 0.05). The statistical analysis excludes the stamen wine, petal wines, and petal ethanol extract.

5. Declaration of Generative AI and AI-assisted Technologies

During the preparation of this manuscript, ChatGPT was used to assist with language translation from Thai to English and sentence rephrasing for clarity. In addition, Grammarly and QuillBot were used for grammar checking, spelling correction, and language polishing.

The use of these tools was limited to improving the quality and readability of the English language. No generative AI tool was used to create or modify the scientific content, including the research design, experimental procedures, data analysis, results, or conclusions. All content is based on original experimental work conducted by the authors, who take full responsibility for the integrity and accuracy of the manuscript.

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