

Exploring the antioxidant and prebiotic potential of fermented Thai jasmine rice beverage: An *in vitro* study

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ABSTRACT

Background: Amazake is a traditional Japanese non-alcoholic rice beverage produced through koji fermentation and recognized as a functional food. Thai jasmine rice (*Oryza sativa* L. KDML 105) is rich in health-promoting nutrients and bioactive compounds, yet no studies have explored the health benefits of fermented Thai jasmine rice beverages.

Objectives: To investigate the health-promoting properties of fermented Jasmine rice amazake (JAS-AMA), focusing on phenolic and flavonoid content, antioxidant capacity, prebiotic effects, and antipathogenic activity against bacteria.

Materials and methods: An *in vitro* study compared JAS-AMA with non-fermented Thai jasmine rice (JAS-SYR) and traditional Japanese amazake (JP-AMA). Total phenolic content, total flavonoid content, DPPH and ABTS antioxidant assays, and prebiotic activity against *Lactobacillus plantarum* (*L. plantarum*) and *Escherichia coli* (*E. coli*) were evaluated using isomaltooligosaccharide (IMO) as a prebiotic reference.

Results: The total phenolic content of JAS-AMA was significantly higher than that of JAS-SYR (28 ± 0.44 compared with $3.0 \pm 0.24 \times 10^{-3}$ mg GAE/ μ L extract, $p < 0.05$), but comparable to that of JP-AMA ($34 \pm 1.3 \times 10^{-3}$ mg GAE/ μ L extract). However, the total flavonoid content of both JAS-AMA and JAS-SYR was significantly lower than JP-AMA (63 ± 6.80 and 12 ± 1.7 , respectively, compared with $128 \pm 22 \times 10^{-7}$ mg CE/ μ L extract, $p < 0.05$). DPPH assay showed comparable antioxidant activity between JAS-AMA and JP-AMA (70.34% and 65.71% inhibition, respectively), both significantly higher than JAS-SYR (20.14% inhibition, $p < 0.05$), whereas ABTS assay revealed significantly lower antioxidant activity in both JAS-AMA and JAS-SYR, lower than JP-AMA (53.57% and 66.05% compared with 95.42% inhibition, $p < 0.05$). The maximum OD600 of *L. plantarum* grown with JAS-AMA (2.0025 ± 0.10) was comparable to that of glucose (1.8050 ± 0.1882) and significantly higher than that of IMO alone (1.4213 ± 0.0925 , $p < 0.05$), suggesting a notable prebiotic effect. No significant differences in *E. coli* growth were observed across any substrate condition ($p > 0.05$).

Conclusion: Jasmine rice amazake demonstrates comparable phenolic content and partial antioxidant activity to traditional Japanese amazake, supporting its potential as a functional beverage and encouraging further development using other rice varieties.

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Introduction

Traditional Japanese diets are widely associated with longevity and good health in the Japanese population. A key element of Washoku is rice as the primary carbohydrate source, commonly consumed alongside a variety of fermented foods produced with *Aspergillus oryzae* (*A. oryzae*) (Koji).^{1,2} One representative fermented product is amazake, a sweet, non-alcoholic rice beverage made by fermenting cooked rice with koji. Traditionally, Japanese white rice (*Oryza sativa japonica*) is used for amazake production, during which koji enzymes promote starch saccharification, resulting in the formation of over 300 compounds, including metabolites derived from Koji.^{3,4} Due to its rich content of fermentation-derived carbohydrates and bioactive compounds, amazake has gained attention as a potential functional food, particularly amid the rapidly expanding global prebiotic market.⁵ Previous studies have identified prebiotic compounds in koji amazake, such as isomaltooligosaccharides (IMO), isomaltotriose, and monohexosyl-ceramides, which have been reported to beneficially modulate intestinal microbiota and improve gastrointestinal health. In addition, amazake contains essential vitamins that contribute to overall human health.^{2,4,6} Despite these recognized benefits, current research has primarily focused on amazake produced from Japanese white rice, leaving a critical knowledge gap regarding how different rice varieties influence fermentation outcomes, prebiotic composition, and functional properties. Therefore, further investigation into amazake is necessary to deepen scientific understanding and support the development of diverse, evidence-based prebiotic products.

Thai jasmine rice (*Oryza sativa* L. KDML 105), along with Japanese white rice (*Oryza sativa japonica*), is one of the two main subspecies of Asian cultivated rice, both of which are staple foods for half of the world's population. These subspecies exhibit distinct structural, biochemical, and physiological characteristics, as evidenced by differences in starch microstructure and protein composition, which influence starch digestibility and metabolic behavior.^{7,8} Such intrinsic compositional differences are likely to affect enzymatic starch breakdown and saccharification efficiency during fermentation with *A. oryzae*. Consequently, variations in rice subspecies may influence the fermentation dynamics and the profile of bioactive compounds produced in amazake. Despite its widespread consumption and economic importance across Asia, Thai jasmine rice remains underexplored as a substrate for fermented products such as amazake.

The objective of this study is to explore the potential health-promoting properties of fermented Thai jasmine rice beverage or Thai jasmine rice amazake. These properties include antioxidant potential, assessed by measuring phenolic content and antioxidant capacity as previously studied in traditional amazake,⁶ and

prebiotic potential, evaluated *in vitro* using selected probiotic and pathogenic bacterial strains to assess the selective effects of isomaltooligosaccharides (IMO), serving as a prebiotic reference for comparison with the amazake candidate. *L. plantarum* was included as a well-established probiotic with documented IMO-mediated growth stimulation, serving as a reference for prebiotic activity.^{4,9} This *in vitro* study has examined the growth of pathogenic bacteria, specifically *E. coli*, which are expected to proliferate poorly on oligosaccharide carbohydrate sources and jasmine rice-based amazake, to assess the antipathogenic potential.¹⁰

Materials and methods

Chemicals

Gallic acid (Hi-media, India, Hi-AR grade), trolox (Sigma-Aldrich, US, AR grade purity ≥98%), folin-ciocalteu reagent (Sigma-Aldrich, US, AR grade purity ≥98%), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, US, AR grade purity ≥98%), diammonium salt of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Hi-media, India, Hi-AR grade), sodium nitrite (NaNO_2) (KEMAUS, Australia, AR grade purity ≥98%), potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$) (Hi-media, India, Hi-AR grade), aluminium chloride (AlCl_3) (KEMAUS, Australia, AR grade purity ≥98%), sodium hydroxide (NaOH) (RCI Labscan, Thailand, AR grade), sodium carbonate (Na_2CO_3) (Hi-media, India, Hi-AR grade), isopropanol (Sigma-Aldrich, US, AR grade purity ≥98%), Isomaltooligosaccharide (IMO) (Wako, Japan, Food grade).

Sample preparation

The amazake used in this study was kindly provided by YoRice Café (Chiang Mai, Thailand), a producer and distributor of YoRice amazake beverages made from Thai jasmine rice (*Oryza sativa* L., KDML 105). The production process involved preparing the koji starter by inoculating steamed Japanese rice (*Oryza sativa japonica*) with *A. oryzae* and incubating it at 40 °C for 3 days. The resulting starter was then mixed with steamed jasmine rice and clean water, followed by 24 hrs of fermentation at 50 °C with continuous stirring. This production process was developed through iterative trial-and-error optimization of the product. The fermented mixture was subsequently pasteurized and distributed.

The modified amazake samples used in this study consisted of three groups: 1) amazake made from 0.5 kg of cooked Thai jasmine rice fermented with 0.5 kg of koji (1:1 ratio) homogenized with 1 liter of drinking water and pasteurized (JAS-AMA), 2) cooked jasmine rice 0.5 kilogram extract mixed with the sucrose syrup low calories 200 mL/kg of jasmine rice homogenized with 1 liter drinking water to replace the sugar that would be created after fermentation, but not subjected to fermentation or pasteurization (JAS-SYR), and 3) a commercial Japanese amazake product prepared from Japanese rice (JP-AMA), with the trade name

withheld to avoid potential impact on product image. All samples were transported at 7 °C and stored at -20 °C until extraction and subsequent analysis. The sample extraction procedure was based on Oguro *et al.*'s study.¹¹ Briefly, 10.0 mL of the sample was centrifuged in a Universal 320 centrifuge (Hettich) at $2,300 \times g$ for 5 min to separate into layers. The solution supernatant was transferred into a 250-mL evaporating flask and dried using a Rotary Evaporator Hel-Vop (Heidolph, Germany) until the sample was completely dry. The dried sample was then dissolved in 1 mL of 50% isopropanol. The sample was then diluted fivefold with distilled deionized water (1:4 sample-to-water ratio). The sample was then filtered with a 0.22-micron Nylon syringe filter, obtaining approximately 4.0-4.5 mL of filtrate. The filtered sample was aliquoted and stored in a clean 1 mL glass vial at -20 °C before analysis

Determination of total flavonoid content (TFC)

The sample extract was determined for total flavonoid content (TFC) using the aluminum chloride method, following Mehmood *et al.*'s modifications.¹² Briefly, 20 µL of the amazake extract was added to a 96-well plate, followed by 80 µL of distilled water and 6 µL of a 5% (w/v) NaNO_2 solution. The mixture was incubated in the dark at room temperature for 6 min. Subsequently, 6 µL of a 10% (w/v) AlCl_3 solution was added, and the mixture was incubated for an additional 6 min under the same conditions. Finally, 40 µL of 1M NaOH was added, and the absorbance was measured at 510 nm. The TFC was expressed as milligrams of catechin equivalents per gram of extract (mg CAE/gm extract).

Determination of total phenolic content (TPC)

The sample extract was determined for total phenolic content (TPC) using the Folin-Ciocalteu method based on Mehmood *et al.* modifications.¹² Briefly, 15 µL of the extract amazake sample and 125 µL of 10% Folin-Ciocalteu reagent were added to a 96-well plate and incubated in the dark at room temperature for 5 min, followed by the addition of 125 µL of 1.89 M Na_2CO_3 solution (w/v). The mixtures were thoroughly mixed and incubated in the dark at room temperature for 90 min. Absorbance was then measured at 725 nm. The TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/gm extract).

Determination of DPPH free radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity was determined using a colorimetric method with trolox as the standard, following the modifications of Zengin *et al.*¹³ A 20 µL volume of the amazake extract was added to a 96-well plate, followed by 180 µL of an 80 µM DPPH methanol solution. The mixtures were thoroughly mixed and incubated in the dark at room temperature for 5 min. Following incubation, the absorbance was measured at 490 nm. The DPPH free radical scavenging activity was calculated using the equation:

$$\text{DPPH \%inhibition} = \frac{[(\text{OD of sample} - \text{OD of blank}) / \text{OD of control}] \times 100}$$

Determination of ABTS free radical scavenging assay

The ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging activity was determined using a colorimetric method with trolox as the standard, based on Re *et al.* modifications.¹⁴ The ABTS solution was prepared by mixing 2 mM ABTS with 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ in a 1:1 ratio, then kept in the dark at room temperature for 16 hrs. Then the ABTS solution was diluted with 50 mM phosphate buffer (pH 7.4) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. A 20 µL volume of the amazake extract was added to a 96-well plate, followed by 200 µL of ABTS solution. The mixtures were thoroughly mixed and incubated in the dark at room temperature for 6 min. Following incubation, the absorbance was measured at 734 nm. The ABTS free radical scavenging activity was calculated using the equation:

$$\text{ABTS \%inhibition} = \frac{[(\text{OD of sample} - \text{OD of blank}) / \text{OD of control}] \times 100}$$

Probiotic growth stimulation and pathogenic inhibition stimulation

Probiotic growth stimulation and pathogenic inhibition were assessed following the method of Rurangwa *et al.*, specifically targeting the probiotic bacterium *L. plantarum* (TISTR-2074) and the pathogenic bacterium *E. coli* (TISTR-527).¹⁵⁻¹⁷ They were grown in modified MRS (wMRS) broth supplemented with mixture of 20 gm/L beef extract and proteose peptone, 5 gm/L yeast malt broth, 1 gm/L tween 20, 1 gm/L citric acid monohydrate, 1 gm/L ammonium acetate, 5 gm/L sodium acetate, 2 gm/L di-sodium hydrogen phosphate, 0.2 gm/L magnesium sulfate anhydrous, and 0.05 gm/L manganese (II) sulfate monohydrate, dissolved in distilled water with content 20 gm/L different substrates (in ratio 2:3 of content wMRS) including glucose (GLU), isomaltoligo-saccharide (IMO), and Thai jasmine rice amazake (The solution was approximated using the phenol-sulfuric acid method compare with glucose 20 gm/L). After semi-anaerobic incubation (sealed with a cotton plug) at 37 °C for 48 hrs, an approximate cell concentration of 3×10^8 cells/mL was obtained in different MRS broths supplemented with various substrates (inoculum: 1% of the broth). For detecting the microbe in broth, we referenced the light wavelengths that should be used from the Montesinos-Cruz and Somerville study.¹⁸ Samples were collected to determine growth or inhibition by monitoring the optical density at 600 nm in the GENESYS™ 180 UV-Vis spectrophotometer (Thermo Scientific, United States). at 600 nm.

Statistical analysis

One-way analysis of variance (ANOVA) followed

by Tukey's test was performed using the Statistical Package for the Social Sciences (SPSS; IBM, United States). Differences with a $p < 0.05$ were considered significant. All data are reported as the means of triplicate measurements $\pm$ standard deviation (SD).

Results

Total phenolic content, total flavonoid content, and antioxidant activity

Standard curves for all four assays demonstrated excellent linearity across the tested concentration ranges (Figure 1). For the TFC assay, catechin was used as the reference standard (0.0004-0.0020 mg/mL),

yielding a linear equation of $y = 73.217x + 0.0007$ with an R^2 of 0.9967. For the TPC assay, gallic acid served as the standard (0.0098-1.2500 mg/mL), with a regression equation of $y = 2.1717x + 0.0466$ and an R^2 of 0.9981. For the DPPH radical-scavenging assay, Trolox was used as the reference standard (0.0020-0.0310 mg/mL), yielding a linear equation of $y = 1842.8x + 18.804$ with an R^2 of 0.9979. Similarly, the ABTS assay standard curve using Trolox (0.0020-0.5000 mg/mL) yielded a regression equation of $y = 128.92x + 5.3052$ with an R^2 of 0.9998. All R^2 values exceeded 0.99, confirming high linearity and reliability for each calibration curve across the measured concentration ranges.

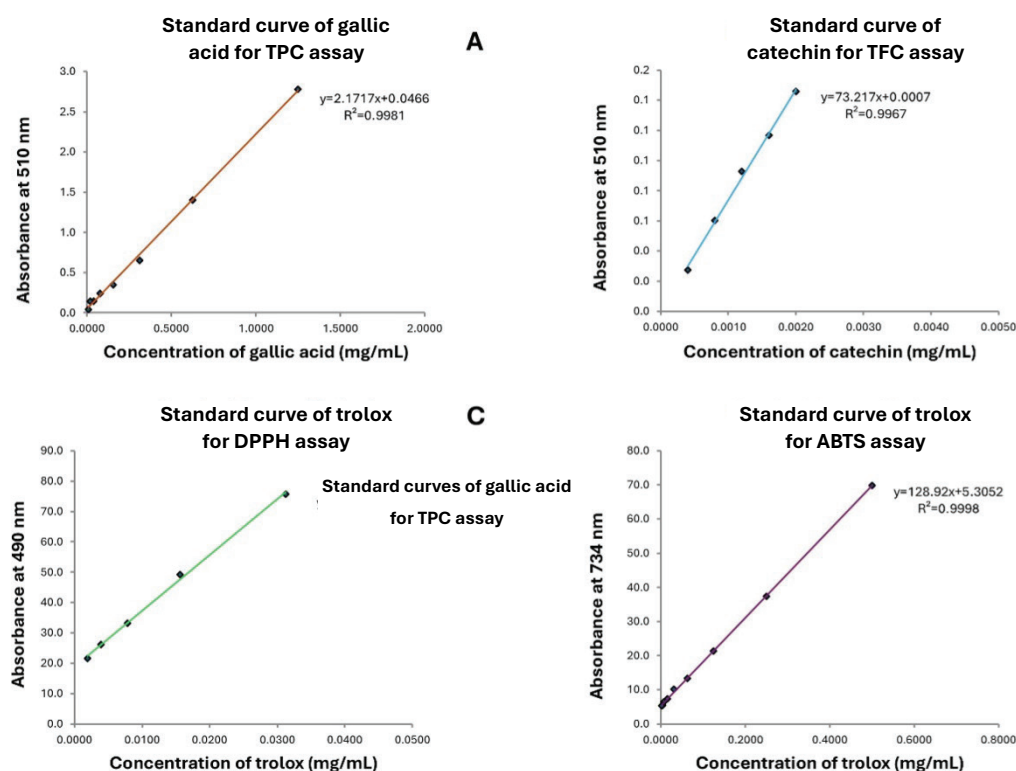


Figure 1. Standard curves for catechin in the TPC assay (A), gallic acid in the TFC assay (B), trolox in the DPPH (C) and ABTS (D) for radical-scavenging assay. Each data point represents the average of triplicate measurements. Linear regression equations and coefficients of determination ($R^2 > 0.99$) are shown for each curve.

TPC and TFC ranked in descending order as JP-AMA > JAS-AMA > JAS-SYR (Figure 2). Koji fermentation significantly enhanced both TPC ($28.33 \pm 0.44 \times 10^{-3}$ mg GAE/ μ L) and TFC ($63.51 \pm 6.80 \times 10^{-7}$ mg CE/ μ L) in JAS-AMA compared to JAS-SYR ($3.09 \pm 0.24 \times 10^{-3}$ mg GAE/ μ L and $12.75 \pm 1.7 \times 10^{-7}$ mg CE/ μ L, respectively, $p < 0.05$), though both remained significantly lower than JP-AMA ($34.6 \pm 1.3 \times 10^{-3}$ mg GAE/ μ L and $128.84 \pm 22 \times 10^{-7}$ mg CE/ μ L, respectively, $p < 0.05$).

For antioxidant activity (Figure 3), DPPH scavenging by JAS-AMA ($70.34 \pm 0.42\%$) was significantly higher than that of JP-AMA ($65.71 \pm 1.16\%$), with both markedly exceeding JAS-SYR ($20.13 \pm 2.22\%$, $p < 0.05$). Conversely, ABTS scavenging followed the order JP-AMA ($96.42 \pm 0.28\%$) > JAS-SYR ($66.05 \pm 0.62\%$) > JAS-AMA ($53.57 \pm 0.89\%$), with all groups significantly different from each other ($p < 0.05$).

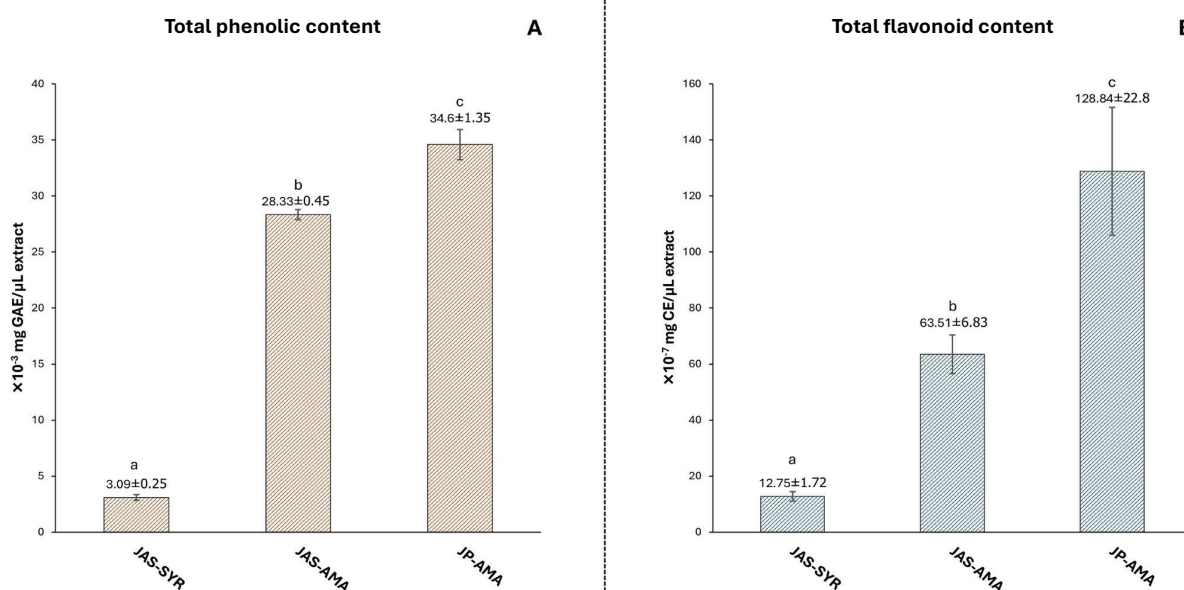


Figure 2. Total phenolic content (A) and total flavonoid content (B) of non-fermented Thai jasmine rice (JAS-SYR), Thai jasmine rice amazake (JAS-AMA), and traditional Japanese amazake (JP-AMA). Values are expressed as mean $\pm$ standard deviation (N=3). TPC is expressed as mg gallic acid equivalents (GAE)/ μ L, and TFC as mg catechin equivalents (CE)/ μ L. Values followed by different lowercase letters are significantly different ($p < 0.05$, Tukey's test).

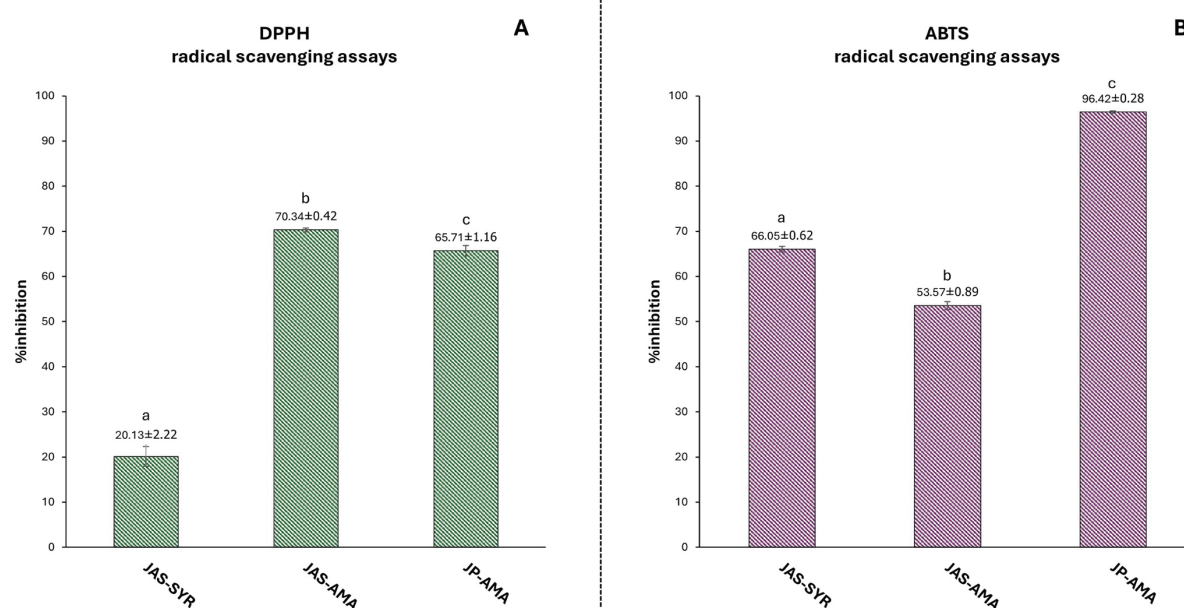


Figure 3. Antioxidant activity of non-fermented Thai jasmine rice (JAS-SYR), Thai jasmine rice amazake (JAS-AMA), and traditional Japanese amazake (JP-AMA) as measured by DPPH (A) and ABTS (B) radical scavenging assays. Values are expressed as mean $\pm$ SD (N=3). Antioxidant activity is reported as a percentage inhibition. Values followed by different lowercase letters are significantly different ($p < 0.05$, Tukey's test).

Probiotic growth stimulation and pathogenic growth inhibition

To evaluate the prebiotic potential of JAS-AMA, the growth of *L. plantarum* and *E. coli* was monitored over 48 hrs by measuring optical density at 600 nm (OD₆₀₀), with the 36-hour time point used for endpoint comparisons (Figure 4). Four substrate conditions were tested for each bacterium: a carbohydrate-free modified

MRS broth as a negative control (LP_wMRS / EC_wMRS), glucose-supplemented MRS broth as a positive control (LP_GLU / EC_GLU), isomaltooligosaccharide-supplemented MRS broth as a prebiotic reference (LP_IMO / EC_IMO), and Thai jasmine rice amazake extract-supplemented MRS broth as the test substrate (LP_JAS_AMA / EC_JAS_AMA).

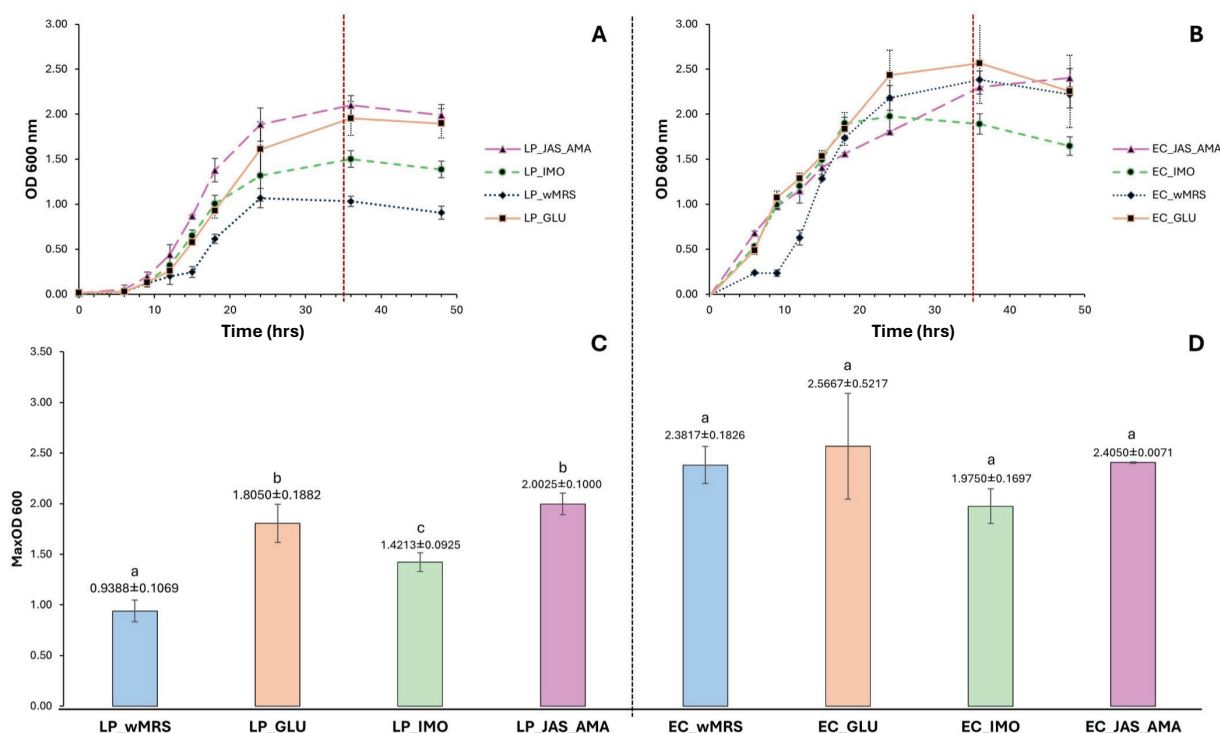


Figure 4. Growth of *L. plantarum* (LP) (A, C) and *E. coli* (EC) (B, D) in the presence of different amazake substrates. (A, B) Optical density (OD₆₀₀) measurements over time in media supplemented with wMRS (control), GLU (glucose), IMO (isomaltooligosaccharide), and JAS-AMA (Thai jasmine rice amazake). The dashed vertical line at 36 hrs indicates the time point used for endpoint comparisons with mean OD₆₀₀ ± SD at 36 hrs. (N=3) below figure (C, D). Values followed by different lowercase letters are significantly different ($p < 0.05$, Tukey's test).

For *L. plantarum*, maximum OD₆₀₀ differed significantly among substrate groups ($p < 0.05$). LP_JAS_AMA (2.0025 ± 0.10) and LP_GLU (1.8050 ± 0.1882) achieved comparable maximum growth and were not significantly different from each other (both group b), while LP_IMO (1.4213 ± 0.0925, group c) and LP_wMRS (0.9388 ± 0.1069, group a) showed progressively lower growth. The finding that LP_JAS_AMA supported *L. plantarum* growth at least as effectively as glucose, and markedly better than IMO alone, suggests a notable prebiotic effect of JAS-AMA on this probiotic strain.

In contrast, no significant differences in maximum OD₆₀₀ were observed among *E. coli* groups ($p > 0.05$), with all groups sharing the same statistical label (group A) and values ranging from EC_IMO (1.9750 ± 0.1697) to EC_GLU (2.5667 ± 0.5217). The inability of JAS-AMA to selectively suppress *E. coli* growth under the tested conditions suggests that the antipathogenic potential of JAS-AMA may not act through direct carbohydrate-

mediated growth inhibition; further investigation into other inhibitory mechanisms, such as antimicrobial metabolites produced during koji fermentation, is warranted.

Discussion

Fermentation by *A. oryzae* releases carbohydrate-degrading enzymes, including α -amylase, xylanase, and glucosidase, that break the cross-links of bound phenolic compounds. Rather than fermentation temperature, which aligns with the optimal activity range (~50 °C) of key *Aspergillus* enzymes,^{19,20} the composition of the rice cell wall likely governs the concentration and types of phenolics released, explaining the observed varietal differences in TPC and TFC between JAS-AMA and JP-AMA.²¹

Antioxidant capacity generally showed a positive association with phenolic content, consistent with previous reports.^{22,23} However, JAS-AMA exhibited

higher DPPH scavenging activity than JP-AMA despite lower TPC, suggesting additional contributions from fermentation-derived compounds such as gamma-oryzanols, tocopherols, and tocotrienols.^{23,24} The DPPH and ABTS assays differ in their sensitivity to antioxidants. The DPPH primarily reflects hydrophobic activity, whereas ABTS captures both hydrophilic and lipophilic antioxidants.²⁵ This distinction is relevant to interpreting the unexpectedly elevated ABTS activity in JAS-SYR, which likely reflects uncharacterized antioxidant constituents in the added syrup rather than phenolic or flavonoid content, representing a key limitation of this study.

Beyond antioxidant activity, phenolic compounds and fermentation-derived oligosaccharides, including isomaltooligosaccharides (IMO), cellobiose, and mannobiose, have been proposed as prebiotic substrates that selectively stimulate beneficial gut bacteria, such as *Lactobacillus* spp., thereby conferring health benefits on the host.²⁶⁻²⁸ In the present study, JAS-AMA supported *L. plantarum* growth to levels comparable to those with glucose and significantly greater than those with IMO alone, suggesting a synergistic prebiotic effect from the combined presence of fermentation-derived oligosaccharides and phenolic compounds. Mechanistically, prebiotic fermentation promotes the production of short-chain fatty acids (SCFAs), including butyrate and propionate, which lower colonic pH and exert anti-inflammatory effects.²⁹

No significant inhibition of *E. coli* growth was observed under any substrate condition ($p>0.05$), suggesting that the antipathogenic potential of JAS-AMA does not operate through carbohydrate-mediated growth restriction alone. Antimicrobial effects are more likely attributable to secondary metabolites produced during koji fermentation, as metabolic profiling of *A. oryzae* has identified coumarins, fatty acids, and quinone compounds with documented activity against *E. coli*.^{30,31} Whether these compounds reach effective concentrations in JAS-AMA warrants further investigation.

Limitations

Several limitations should be acknowledged in this study. Firstly, the chemical composition of the sucrose syrup used in JAS-SYR preparation was not characterized, which prevents a full interpretation of the unexpectedly elevated ABTS radical-scavenging activity observed in that sample, as unquantified antioxidant constituents in the syrup may have independently contributed to this response. Secondly, the study encompassed only three sample types and did not involve in-depth characterization, limiting the generalizability of the findings across a wider range of rice varieties and fermentation conditions. Thirdly, the DPPH assay, being conducted primarily in organic solvents, may have underrepresented the contribution of hydrophilic antioxidants, and the specific enzymatic

mechanisms and fermentation parameters responsible for the observed differences between JP-AMA and JAS-AMA were not systematically investigated. Lastly, the prebiotic assessment was conducted exclusively *in vitro*, and the observed growth stimulation of *L. plantarum* and the lack of significant inhibition of *E. coli* under these conditions may not fully reflect the complex ecological interactions that occur within the human gastrointestinal tract, where additional host-derived and microbial factors may modulate the antipathogenic response. These limitations should be considered when interpreting the findings of the present study and highlighting important directions for future research.

Conclusion

In conclusion, this study provides new evidence supporting Thai jasmine rice-based amazake as a novel functional beverage with combined antioxidant and prebiotic properties. To our knowledge, this work demonstrates that koji fermentation of Thai jasmine rice significantly increases phenolic and flavonoid contents, thereby enhancing antioxidant capacity. The observed promotion of *L. plantarum* growth, comparable to that of glucose and superior to IMO alone, highlights the prebiotic potential of jasmine rice amazake in selectively stimulating beneficial gut bacteria. Although significant inhibition of *Escherichia coli* was not demonstrated under the *in vitro* conditions tested, the antipathogenic potential of JAS-AMA warrants further investigation, particularly regarding fermentation-derived antimicrobial metabolites such as coumarins, fatty acids, and quinones.

These findings extend current knowledge beyond traditionally studied Japanese rice-based amazake and establish Thai jasmine rice as a promising alternative substrate for prebiotic beverage development.

Furthermore, the results suggest that varietal diversity within *A. oryza*, including white and pigmented jasmine rice varieties, may differentially influence fermentation outcomes and bioactive compound profiles. This opens new avenues for targeted fermentation strategies to maximize health benefits. Future clinical validation is warranted to confirm the impacts on gut health, immune modulation, and overall well-being, thereby supporting the translation of jasmine rice amazake into evidence-based functional food applications.

Availability of data and materials

The data are available from the corresponding author on reasonable request.

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The YoRice amazake samples were kindly provided by YoRice Café.

CRedit authorship contribution statement

Ran Kitkangplu: conceptualization, methodology, resources, validation, laboratory operator, formal analysis, visualization, writing: original draft and edit; **Krongpong Monpengpinij:** conceptualization, validation, formal analysis, project administration, writing: original draft; **Kongkiat Kespechara:** conceptualization, resources, validation, formal analysis, project administration, writing: original draft; **Niwed Kullawong** and **Woottichai Nachaiwieng:** conceptualization, methodology, resources, validation, formal analysis, project administration.

Conflict of interest

The authors declare no competing interests.

Declaration of AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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References

- [1] Gabriel AS, Ninomiya K, Uneyama H. The role of the Japanese traditional diet in healthy and sustainable dietary patterns around the world. *Nutrients*. 2018; 10(2): 173. doi: 10.3390/nu10020173.
- [2] Hamajima H, Matsunaga H, Fujikawa A, et al. Japanese traditional dietary fungus koji *Aspergillus oryzae* functions as a prebiotic for *Blautia coccoides* through glycosylceramide: Japanese dietary fungus koji is a new prebiotic. *SpringerPlus*. 2016; 5(1): 1321. doi: 10.1186/s40064-016-2950-6.
- [3] Hamajima H, Fujikawa A, Yamashiro M, et al. Chemical analysis of the sugar moiety of monohexosylceramide contained in koji, Japanese traditional rice fermented with *Aspergillus*. *Fermentation*. 2016; 2(1): 2. doi: 10.3390/fermentation2010002.
- [4] Kurahashi A. Ingredients, functionality, and safety of the Japanese traditional sweet drink amazake. *J Fungi (Basel)*. 2021; 7(6): 469. doi: 10.3390/jof7060469.
- [5] hoareaup. The prebiotics market forecast to 2023 and beyond [Internet]. Gnosis by Lesaffre; 2022 Mar 9 [cited 2025 Dec 27]. Available from: <https://gnosisbylesaffre.com/blog/the-prebiotics-market-forecast-to-2023-and-beyond/>.
- [6] Oguro Y, Nishiwaki T, Shinada R, Kobayashi K, Kurahashi A. Metabolite profile of koji amazake and its lactic acid fermentation product by *Lactobacillus sakei* UONUMA. *J Biosci Bioeng*. 2017; 124(2): 178-83. doi: 10.1016/j.jbiosc.2017.03.011.
- [7] Schwanz Goebel JT, Kaur L, Colussi R, Elias MC, Singh J. Microstructure of indica and japonica rice influences their starch digestibility: a study using a human digestion simulator. *Food Hydrocolloids*. 2019; 94: 191-8. doi: 10.1016/j.foodhyd.2019.02.038.
- [8] Yang Y, Zhu K, Xia H, Chen L, Chen K. Comparative proteomic analysis of indica and japonica rice varieties. *Genet Mol Biol*. 2014; 37(4): 652-61. doi: 10.1590/S1415-47572014005000015.
- [9] Soto C. Effect of isomaltooligosaccharide and gentiooligosaccharide on the growth and fatty acid profile of *Lactobacillus plantarum*. *Electron J Biotechnol [Internet]*. 2013 [cited 2025 Sep 10];16(4). Available from: <https://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v16n4-9>.
- [10] Chantorn S, Piyapittayanun C, Dangpram P. Bioconversion of agricultural wastes to mannooligosaccharides and their prebiotic potential. *Chiang Mai J Sci*. 2018; 45(1): 60-7.
- [11] Oguro Y, Nishiwaki T, Shinada R, Kobayashi K, Kurahashi A. Metabolite profile of koji amazake and its lactic acid fermentation product by *Lactobacillus sakei* UONUMA. *J Biosci Bioeng*. 2017; 124(2): 178-83. doi: 10.1016/j.jbiosc.2017.03.011.
- [12] Mehmood A, Javid S, Khan MF, Ahmad KS, Mustafa A. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chem*. 2022; 16(1): 64. doi: 10.1186/s13065-022-00858-2.
- [13] Zengin G, Sarikurkcu C, Uyar P, et al. *Crepis foetida* L. subsp. *rhoeadifolia* (Bieb.) Celak. as a source of multifunctional agents: cytotoxic and phytochemical evaluation. *J Funct Foods*. 2015; 17: 698-708. doi: 10.1016/j.jff.2015.06.041.
- [14] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999; 26(9-10): 1231-7. doi: 10.1016/S0891-5849(98)00315-3.
- [15] Rurangwa E, Laranja JL, Van Houdt R, et al. Selected nondigestible carbohydrates and prebiotics support the growth of probiotic fish bacteria mono-cultures in vitro. *J Appl Microbiol*.

- 2009; 106(3): 932-40. doi: 10.1111/j.1365-2672.2008.04034.x.
- [16] Kerdsup P, Hattayapichat P, Silva JL, Tantratian S. Survival of potential probiotic isolated from fermented tea leaf and encapsulated in multilayer beads stored in makiang (*Cleistocalyx nervosum* var. *paniala*) juice. *Food Biosci.* 2022; 50: 102015. doi: 10.1016/j.fbio.2022.102015.
- [17] Saelim K. Transfer of *Escherichia coli* TISTR527 from surface to fresh-cut cantaloupe [Internet]. Published online 2020. doi: 10.14458/RSU.RES.2020.223.
- [18] Montesinos-Cruz V, Somerville GA. Shining a light on spectrophotometry in bacteriology. *Antibiotics.* 2024; 13(12): 1164. doi: 10.3390/antibiotics13121164.
- [19] Yan H. Biocatalytic aldol addition reactions by lipase from *Aspergillus oryzae*. *Chiang Mai J Sci.* 2025; 52(2). doi: 10.12982/CMJS.2025.019.
- [20] Kumar S, Tissopi T, Mutturi S. The successful synthesis of industrial isomaltooligosaccharides lies in the use of transglycosylating -glucosidases: a review. *Carbohydr Polym Technol Appl.* 2023; 5: 100325. doi: 10.1016/j.carpta.2023.100325.
- [21] Saharan P, Sadh PK, Singh Duhan J. Comparative assessment of effect of fermentation on phenolics, flavanoids and free radical scavenging activity of commonly used cereals. *Biocatal Agric Biotechnol.* 2017; 12: 236-40. doi: 10.1016/j.bcab.2017.10.013.
- [22] Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* 2004; 74(17): 2157-84. doi: 10.1016/j.lfs.2003.09.047.
- [23] Choi Y, Jeong HS, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem.* 2007; 103(1): 130-8. doi: 10.1016/j.foodchem.2006.08.004.
- [24] Xu Z, Hua N, Godber JS. Antioxidant activity of tocopherols, tocotrienols, and -oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis(2-methyl propionamide) dihydrochloride. *J Agric Food Chem.* 2001; 49(4): 2077-81. doi: 10.1021/jf0012852.
- [25] Rumpf J, Burger R, Schulze M. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *Int J Biol Macromol.* 2023; 233: 123470. doi: 10.1016/j.ijbiomac.2023.123470.
- [26] Plamada D, Vodnar DC. Polyphenols—gut microbiota interrelationship: a transition to a new generation of prebiotics. *Nutrients.* 2021; 14(1): 137. doi: 10.3390/nu14010137.
- [27] Sawangwan T, Saman P. Prebiotic synthesis from rice using *Aspergillus oryzae* with solid state fermentation. *Agric Nat Resour.* 2016; 50(4): 227-31. doi: 10.1016/j.anres.2016.02.004.
- [28] Fuloria S, Mehta J, Talukdar MP, et al. Synbiotic effects of fermented rice on human health and wellness: a natural beverage that boosts immunity. *Front Microbiol.* 2022; 13: 950913. doi: 10.3389/fmicb.2022.950913.
- [29] Davani-Davari D, Negahdaripour M, Karimzadeh I, et al. Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods.* 2019; 8(3): 92. doi: 10.3390/foods8030092.
- [30] Son SY, Lee S, Singh D, Lee NR, Lee DY, Lee CH. Comprehensive secondary metabolite profiling toward delineating the solid and submerged-state fermentation of *Aspergillus oryzae* KCCM 12698. *Front Microbiol.* 2018; 9: 1076. doi: 10.3389/fmicb.2018.01076.
- [31] Tu Z, Choi D, Chen Y, Yu JH, Huynh TN. The food fermentation fungus *Aspergillus oryzae* is a source of natural antimicrobials against *Listeria monocytogenes*. *J Dairy Sci.* 2025; 108(4): 3444-54. doi: 10.3168/jds.2024-25719.