A Study of the Chemical and Microbial Properties of Kombucha Fermented from Local Mushrooms — Hed Kor (*Russula* sp.), Hed Kai-kiew (*Russula* sp.), and Hed Mun Pu (*Cantharellus cibarius*) —, Cultivated in Mahasarakham, Thailand

Wilawan Boonsupa* & Yuwadee Insumran

Department of Biology, Faculty of Science and Technology, Rajabhat Mahasarakham University, Mahasarakham, 44000 Thailand

Abstract

This study aimed to develop kombucha beverages from two wild mushroom species, *Russula* sp. (locally known as Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* (locally known as Hed Mun Pu), with a focus on evaluating their chemical properties, antioxidant potential, microbial content, and nutritional composition. The kombucha starter culture (SCOBY) used in this study was purchased from Northlandtea, Nonthaburi, Thailand. Chemical analyses included quantification of acetic acid, glucose, and fructose using High-Performance Liquid Chromatography (HPLC). Antioxidant activity was assessed via the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay, while total flavonoids and total phenolics were determined using the aluminum chloride colorimetric and Folin-Ciocalteu methods, respectively. Microbial counts were evaluated using the pour plate technique on Sabouraud Dextrose Agar (SDA) for yeast and mold, and Plate Count Agar (PCA) for bacteria. On the final day of fermentation, kombucha derived from *Russula* sp. (Hed Kai-Kiew) exhibited the lowest levels of soluble solids (3.00°Brix), glucose (2.38%), and fructose (4.08%), while showing the highest acetic acid content at 0.82%. The reduced sugar levels in Hed Kai-Kiew kombucha suggest that glucose and fructose were actively metabolized by fermentative microbes, thereby accelerating organic acid production and contributing to its distinctive acidic profile. Such sugar utilization highlights the positive functional role of Hed Kai-Kiew as a substrate that promotes efficient microbial activity during fermentation. Kombucha from *C. cibarius* showed the highest antioxidant activity (320.41 mg/mL). In contrast, Hed Kai-Kiew kombucha had the highest total phenolic content (441.52±3.74 mg/L), and Hed Kor kombucha exhibited the highest total flavonoid concentration (177 µg/mL). On day 15, Hed Kor kombucha had the highest yeast and mold count (2.50×10^{14} CFU/mL), and Hed Kai-Kiew kombucha showed the highest bacterial count (9×10^{8} CFU/mL). These findings demonstrate the potential of wild mushroom-based kombucha as a novel functional beverage with diverse nutritional and bioactive properties.

Keywords: Antioxidant activity, Kombucha, *Russula* sp., *Cantharellus cibarius*

*** Corresponding Author**
e-mail: wilawan.wi@rmu.ac.th.

Introduction

Kombucha is a fermented beverage produced through the symbiotic activity of bacteria and yeast (SCOBY: Symbiotic Culture of Bacteria and Yeast), which metabolize sugars into a variety of bioactive compounds (Jayabalan et al., 2014). Typically, the fermentation involves inoculating a solution of tea and sugar with SCOBY under aerobic conditions. The resulting beverage is known for its distinctive balance of sourness and fruity aroma. With a growing global interest in functional foods and gut health, fermented products like kombucha are increasingly popular due to their potential to enhance probiotic content and promote health benefits (Marco et al., 2021).

Kombucha is considered a traditional drink that has rapidly gained popularity worldwide (Jayabalan et al., 2014; Marsh et al., 2014). Several studies have documented its potential health benefits, including antimicrobial, antioxidant, anticancer, antidiabetic, anti-inflammatory, and cholesterol-lowering activities (Chakravorty et al., 2016; Villarreal-Soto et al., 2018). During fermentation, microbial metabolism of polyphenols in tea leads to the formation of biologically active compounds (Jayabalan et al., 2014). The fermentation parameters such as duration, initial sugar concentration, microbial composition of the SCOBY, and temperature significantly influence the bioactive content and sensory qualities of kombucha (Marsh et al., 2014; Villarreal-Soto et al., 2018).

The fermentation process is characterized by a dynamic microbial ecosystem primarily composed of acetic acid bacteria and yeast. Yeasts secrete extracellular invertase enzymes that hydrolyze sucrose into glucose and fructose. These monosaccharides serve as substrates for both yeast and bacteria. While yeast ferments the sugars to ethanol, glycerol, and carbon dioxide, acetic acid bacteria oxidize ethanol into acetic acid and further produces glucuronic and gluconic acids, contributing to the decrease in pH. Additionally, the bacteria synthesize bacterial cellulose, forming a pellicle layer on the surface of the liquid. This acidic environment helps inhibit the growth of pathogenic organisms, making kombucha a naturally preserved and probiotic-rich beverage without requiring pasteurization (Zhao et al., 2025; Sinir et al., 2019).

Mushrooms, belonging to the fungal kingdom, are filamentous organisms that form fruiting bodies consisting of a cap, stem, gills, and occasionally a protective veil. These structures emerge from a dense network of mycelium, which typically remains embedded in the substrate. While the mycelium is not usually consumed, the fruiting body is widely appreciated for its nutritional and medicinal properties. In recent years, interest has grown in the utilization of wild and edible mushrooms in functional food applications due to their high content of bioactive compounds such as polysaccharides, polyphenols, and flavonoids (Sknepnek et al., 2021). Mushrooms contain a high amount of protein, while carbohydrates constitute the main components, approximately 35–70%, including digestible and nondigestible forms (On-Nom et al., 2023).

Recent studies have highlighted the potential of kombucha fermented with medicinal mushrooms to enhance its bioactive profile. For instance, kombucha prepared with *Ganoderma lucidum* powder has been reported to exhibit improved antioxidant activity and higher polysaccharide content compared to traditional tea-based kombucha (Elfirta et al., 2024). Recent research has increasingly focused on the sensory properties of kombucha enriched with edible and medicinal mushrooms. For example, a study on kombucha prepared with truffles (*Tuber spp.*) demonstrated that mushroom supplementation influenced not only the physicochemical and biochemical parameters but also contributed key sensory attributes, including an aromatic profile mainly composed of esters (fruity notes) that could be attributed to the freshly fermented kombucha beverage. These compounds, especially some esters or acetic acid, could be used as potential marker compounds for fermentation control in kombuchas (Morales et al., 2024). These findings highlight the importance of sensory evaluation when developing mushroom-based kombucha, as consumer acceptance is directly linked to flavor and aroma profiles (Morales et al., 2024).

Russula species, including Hed Kor and Hed Kai-Kiew, are widely found in deciduous forests of Thailand and are valued for their nutritional and medicinal properties. They are ectomycorrhizal fungi forming symbiotic associations with forest trees, contributing to ecosystem balance while providing food resources for local communities. Traditionally, Russula mushrooms are collected during the rainy season and used in local cuisine due to their unique flavor and texture, despite some species having mild bitterness that is reduced after cooking (Sanmee et al., 2003). In addition to their nutritional value (rich in protein, dietary fiber, and essential minerals), Russula species have recently been reported to possess potential health-promoting properties, such as lipase and α-glucosidase inhibitory activities, which may contribute to obesity and diabetes prevention (Pongkunakorn et al. 2017).

Hed Mun Pu (*Cantharellus cibarius*), known as the golden chanterelle, is another ectomycorrhizal mushroom highly prized for its fruity aroma and firm texture. It is seasonally available in northern and northeastern Thailand and commands high market prices. Nutritionally, it provides considerable amounts of carbohydrates, proteins, and essential micronutrients, and it is appreciated both as a food source and for its potential medicinal benefits (Sanmee et al., 2003).

This study explores the development of kombucha using two species of wild mushrooms, *Russula* sp. and *Cantharellus cibarius* with the aim of enhancing the nutritional value and functional properties of kombucha beverages. The research focuses on evaluating chemical composition, antioxidant capacity and microbial content to assess the feasibility of integrating these mushrooms into novel health-promoting probiotic drinks. The novelty of this study lies in integrating Thai wild mushrooms into kombucha production for the first time, thereby diversifying both the raw materials and the functional properties of the beverage. This approach not only introduces unique local biodiversity into functional food development but also provides scientific evidence for the health-promoting potential of underutilized edible mushrooms.

The main objective of this study was to develop kombucha beverages using two wild mushrooms species, *Russula* sp. (Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius*, to enhance the functional and nutritional properties of traditional kombucha. Specific objectives included: (1) To evaluate the chemical properties of mushroom-based kombucha, including acetic acid and sugar content (glucose and fructose) using High-Performance Liquid Chromatography (HPLC), (2) to assess antioxidant activity, total phenolic content, and total flavonoid content of the kombucha samples, and (3) to determine the microbial profile of kombucha during fermentation, focusing on yeast, mold, and bacterial populations.

Materials and methods

1. Chemicals and reagents

All reagents and solvents used in this study were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) and anhydrous aluminum chloride were obtained from Sigma–Aldrich (Germany). Gallic acid standard was purchased from Carlo erba (Italy), and Folin-Ciocalteu reagent was supplied by Merck (Germany). Sodium carbonate (anhydrous) and sodium nitrate were obtained from Kemaus (Australia). Plate Count Agar (PCA) and Sabouraud Dextrose Agar (SDA) were sourced from Himedia (India).

2. Raw materials

Russula sp. (locally known as Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* mushrooms were collected from a local forest in Maha Sarakham Province, Thailand. The conditioning of dry mushrooms was modified from Elfirta et al. (2024). The mushrooms were dried at 75°C for 5 h using a hot air oven, ground into fine powder, and stored in airtight containers until use. A commercial kombucha SCOBY and starter culture (200 mL) were obtained from Northlandtea (Nonthaburi, Thailand). According to the manufacturer, the SCOBY was produced using USDA-certified organic black tea. To minimize batch-to-batch variation, a mother culture was prepared in the laboratory and used as the inoculum for all experimental fermentations throughout the study.

(a) (b) (c)

Fig 1. Size, shape and color of fresh edible Thai local mushrooms used in this study, which included (a) Hed Kor (*Russula* sp.), (b) Hed Kai-Kiew (*Russula* sp.), (c) Hed Mun Pu (*Cantharellus cibarius*)

3. Mushroom kombucha preparation

The kombucha fermentation process was modified based on a previously described method (Boonsupa & Insumran, 2025). Briefly, 10 g of dried mushroom powder (moisture content 11.44±0.43%, determined after drying at 75°C for 5 h) was infused in 1 L of boiling water (80°C) for 15 min in a 3 L sterilized glass vessel. Subsequently, 70 g of sucrose was dissolved in the hot infusion. Once cooled to room temperature, 40 g of SCOBY was added. The vessel was covered with sterile cheesecloth and fermented at 30°C for 15 d.

4. Chemical analysis

Samples were centrifuged and filtered through a 0.45 µm membrane prior to HPLC analysis. Quantification of glucose, fructose, ethanol, and acetic acid was performed using a Shimadzu HPLC-RID system with an Aminex HPX-87H column 300 mm × 7.8 mm (Bio-Rad, USA) and a cation exchange pre-column. The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min, column temperature 45°C, and injection volume 20 µL. Calibration curves ($R^2>0.99$) were established using standard solutions for quantification.

5. Antioxidant activity (DPPH Assay)

The antioxidant capacity was determined using the DPPH radical scavenging assay as described by Brand-Williams et al. (1995), with minor modifications. Equal volumes (5 mL) of kombucha sample and 0.1 mM DPPH solution (in ethanol) were mixed and incubated in the dark at room temperature for 20 min. Absorbance was measured at 517 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Japan). Results were expressed as mg α-tocopherol equivalents/mL of the sample.

6. Total phenolic content (TPC)

The total phenolic content was assessed using the Folin–Ciocalteu method following Singleton and Rossi (1965). 1 mL of the sample was mixed with 9.5 mL of distilled water, followed by 0.5 mL of Folin–Ciocalteu reagent and 2 mL of 10% sodium carbonate. After incubation at room temperature for 30 min, absorbance was recorded at 765 nm. TPC was expressed as mg gallic acid equivalents (GAE)/L.

7. Total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method as described by Zhishen et al. (1999). The reaction mixture consisted of 1 mL of the sample, 4 mL ethanol, 0.3 mL 5% $NaNO_2$, and 0.3 mL 10% $AlCl_3$. After 6 min, 2 mL of 1.0 M NaOH was added, and the total volume adjusted to 10 mL with 70% ethanol. After 15 min at room temperature, absorbance was measured at 510 nm. TFC was expressed as µg rutin equivalents (RUE)/mL.

8. Microbiological analysis

Microbial enumeration was performed on 3 mushroom kombuchas on Day 1 and Day 15 of fermentation. Serial dilutions were plated using the pour plate technique on PCA and SDA media. Plates were incubated at 37°C for 48 h (PCA) and 72 h (SDA), and colony counts were recorded as Log_{10} CFU/mL.

9. Statistical analysis

All experiments were performed in triplicate using independent fermentation batches (2 L in 3 L vessels). Results were presented as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA with SPSS software version 22.0 (IBM, USA), and statistical significance was set at $p<0.05$.

Results and discussion

1. Chemical properties of kombucha fermented from Hed Kor, Hed Kai-Kiew, and *Cantharellus cibarius* Mushrooms

The chemical properties of kombucha fermented from Hed Kor, Hed Kai-Kiew, and *C. cibarius* were monitored during the fermentation process using commercial SCOBY (Symbiotic Culture of Bacteria and Yeast) as the inoculum. The chemical composition was evaluated every 3 d throughout a 15-d fermentation period (days 0, 3, 6, 9, 12, and 15).

1.1 pH values

The pH values of kombucha made from all 3 mushroom types decreased over the fermentation period. On day 0, the initial pH values were 2.87±0.01 for Hed Kor kombucha, 3.30±0.01 for Hed Kai-Kiew, and 2.59±0.01 for *C. cibarius*. By day 15, the pH values had decreased to 2.20±0.18, 2.48±0.01, and 2.29±0.01, respectively (Fig. 2a). This trend indicates acid production during fermentation, primarily due to the conversion of ethanol to acetic acid by acetic acid bacteria (AAB), which increases the concentration of organic acids and decreases pH. According to Sittisart (2024), who investigated the quality and fungal community of Kombucha fermented with hemp leaves and milky mushroom flour (*Calocybe indica*), the resulting pH decreased in kombucha produced with the addition of hemp leaves and milky mushroom flour fermented for 21 d (pH value = 3.23).

1.2 Total soluble solids (TSS)

The total soluble solids (TSS), expressed in degrees Brix (°Brix), were monitored throughout the 15-d fermentation period. A general decreasing trend in TSS was observed across all kombucha samples, indicating the consumption of sugars by the symbiotic culture of bacteria and yeast (SCOBY) during fermentation. Among the samples, kombucha prepared from *Russula sp.* (Hed Kai-Kiew) exhibited the lowest TSS at the end of fermentation, measuring 3.00°Brix. This reduction reflects the efficient microbial metabolism of sucrose and monosaccharides. In contrast, kombucha from *Cantharellus cibarius* retained a slightly higher TSS value of 3.5°Brix on day 15, which may be attributed to slower sugar utilization. The reduction in TSS corresponds well with the decrease in glucose and fructose levels and the increase in acetic acid content observed during the fermentation process. According to Sittisart (2024), who investigated the total soluble solids of kombucha fermented with hemp leaves during 21 d, the results showed that TSS decreased during fermentation and that the final day TSS value was 7°Brix.

1.3 Fructose content

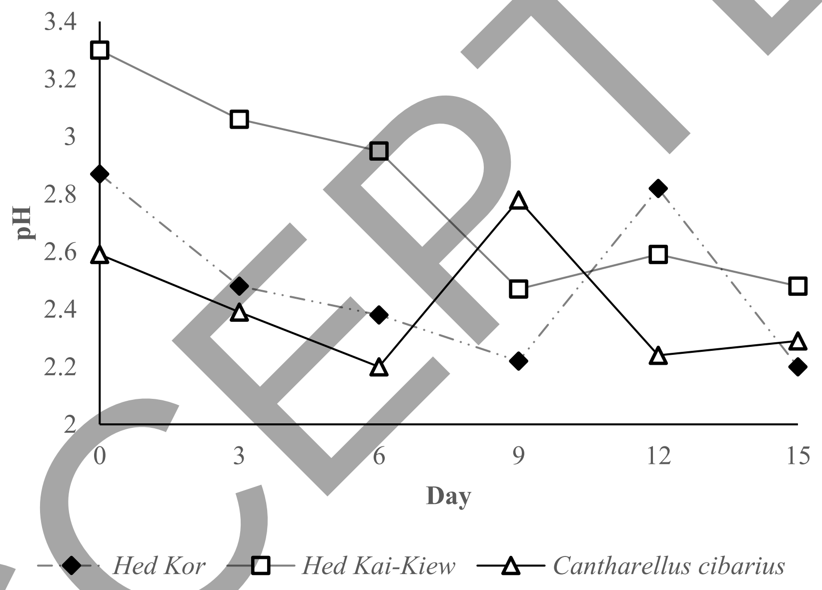
Fructose concentrations in kombucha samples varied during fermentation. Hed Kor kombucha showed a peak on day 0 (7.82±2.54%) and decreased by day 3, then increased again by day 15 (5.20±0.76%). Hed Kai-Kiew kombucha exhibited a rise until day 6 (5.00±0.18%) before declining, with the lowest fructose concentration on day 15 (4.08±0.76%). *C. cibarius* kombucha showed the highest fructose levels on day 12 (6.98±0.78%) (Fig. 2c). The results correspond with the trend of decreasing total soluble solids, especially in Hed Kai-Kiew kombucha. In this study, fructose concentrations varied depending on the mushroom substrate and the stage of fermentation. Similar fluctuations in sugar utilization have been reported in kombucha prepared with *Wolffia arrhiza* (duckweed), where fructose and glucose levels decreased during fermentation due to microbial metabolism, followed by partial recovery at later stages (Boonsupa & Insumran, 2025). These results support the observation that dynamic changes in sugar profiles are a common feature of kombucha fermentation, reflecting the sequential activities of yeasts and acetic acid bacteria.

1.4 Glucose content

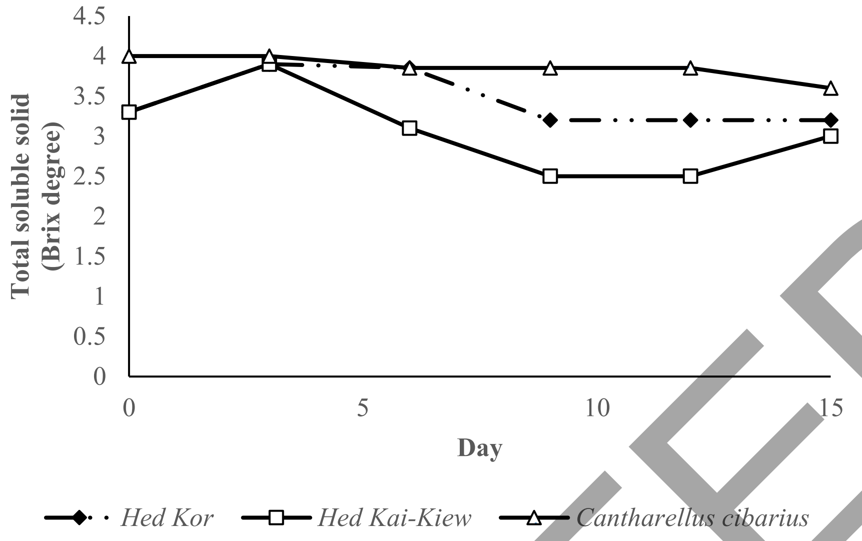
Changes in glucose content during fermentation were also monitored. Hed Kai-Kiew kombucha showed a sharp decrease, reaching the lowest level on day 15 (2.38±0.14%). Hed Kor and *C. cibarius* kombucha showed more gradual decreases. *C. cibarius* kombucha retained relatively higher glucose throughout fermentation (2.84±0.07% on day 15). These results correlate with the overall sugar utilization pattern (Fig. 2d). As Sknepnek et al. (2021) studied the glucose content during the 11-d fermentation of *Coriolus versicolor* and *Lentinus edodes* mushroom kombucha, it was found that the glucose content decreased throughout the fermentation period.

1.5 Acetic acid content

The acetic acid concentration increased with fermentation time. On day 15, Hed Kai-Kiew kombucha had the highest acetic acid content (0.82±0.06%), followed by *C. cibarius* (0.34±0.08%), and Hed Kor (0.33±0.04%) (Fig. 2e). The increase in acetic acid concentration is a result of ethanol oxidation by AAB. During early fermentation, yeast hydrolyzes sucrose into glucose and fructose, which are subsequently converted into ethanol and organic acids, creating favorable conditions for AAB growth and acid production (Dufresne & Farnworth, 2000). According to Sknepnek et al. (2021), who investigated total acids of *Coriolus versicolor* and *Lentinus edodes* mushroom kombucha, the resulting total acids increased in kombucha fermented for 11 d (CV = 35 g/L and LE = 25 g/L).

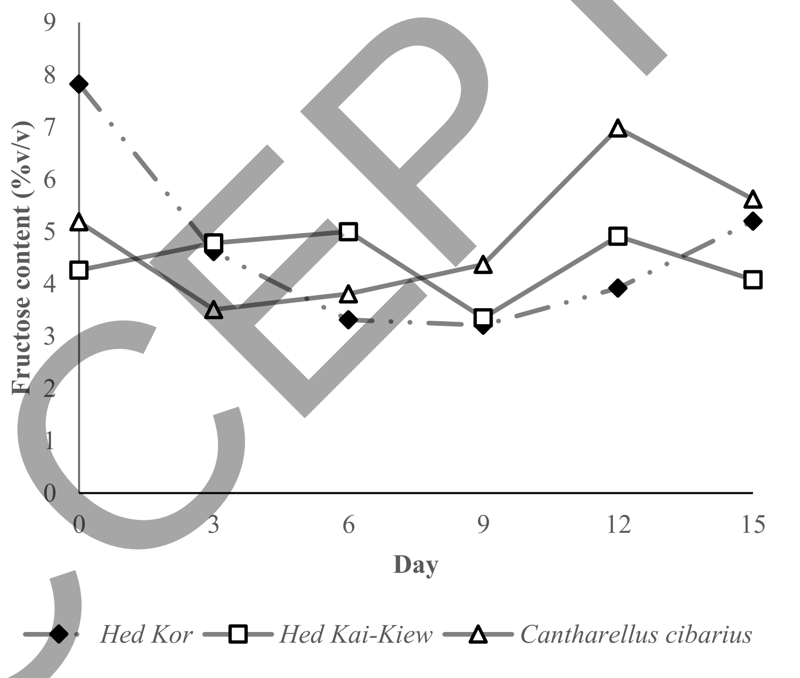


(a)



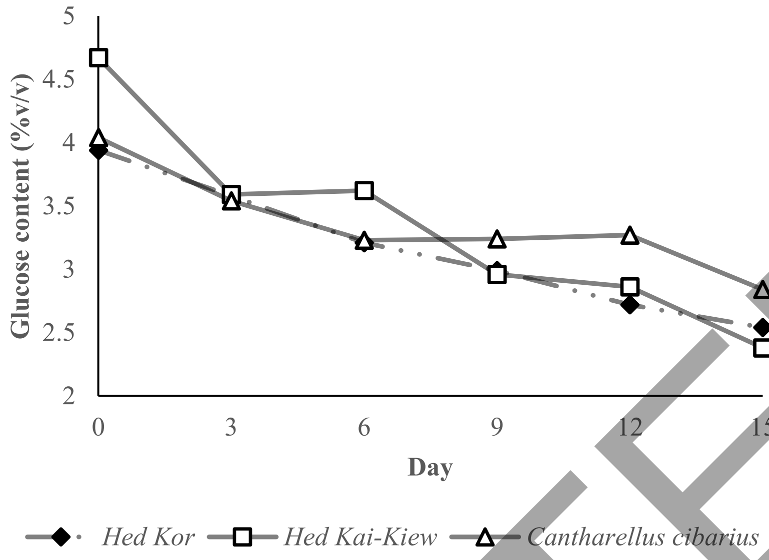
Total soluble solid (Brix degree)
Day
Hed Kor
Hed Kai-Kiew
Cantharellus cibarius

(b)



Fructose content (%v/v)
Day
Hed Kor
Hed Kai-Kiew
Cantharellus cibarius

(c)



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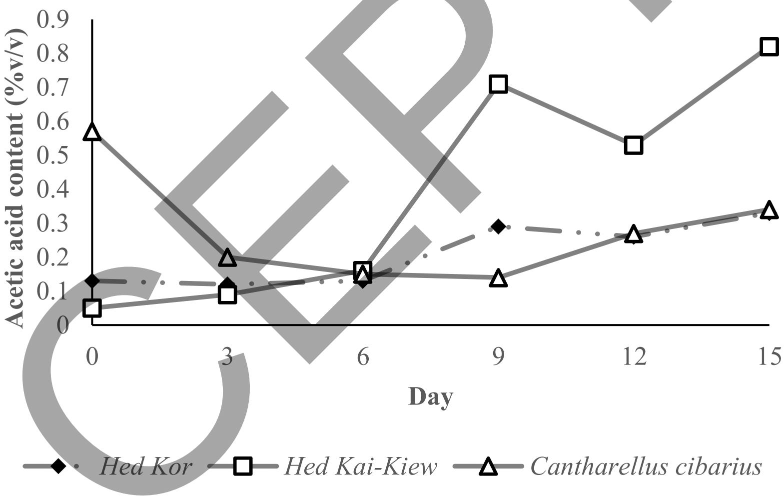


(e)

Fig 2. Changes in chemical properties of 2 wild mushroom species, *Russula* sp (locally known as Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* kombucha during fermentation: a) pH, b) Total soluble solid, c) fructose content, d) glucose content, and e) Total acetic acid

2. Characterization of bioactive compounds

2.1 Antioxidant activity of mushroom kombucha

The antioxidant activity of kombucha samples prepared from *Russula* sp. (Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* was evaluated on Day 15 of fermentation using the DPPH assay. The kombucha from *C. cibarius* exhibited the highest antioxidant activity at 320.41±2.23 mg α-tocopherol equivalents/mL (mg α-TE/mL), followed by Hed Kai-Kiew (124.62±1.00 mg α-TE/mL) and Hed Kor (104.83±1.88 mg α-TE/mL), as shown in Table 1. These findings align with those of Kanyarat et al. (2025), who reported that caffeinated and non-caffeinated coffee kombucha fermented for 12 d exhibited varying antioxidant activities. The highest antioxidant activity was found in caffeinated coffee kombucha. (27.25±0.24 mg/mL).

2.2 Total phenolic content (TPC)

The total phenolic content (TPC) of kombucha samples was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents/L (mg GAE/L). Among the samples, kombucha from Hed Kai-Kiew exhibited the highest TPC at 441.52±3.74 mg GAE/L, followed by Hed Kor (328.29±2.49 mg GAE/L) and *C. cibarius* (223.29±2.07 mg GAE/L), as presented in Table 1. These results correspond with the findings of Edo et al. (2025), who emphasized the crucial role of phenolic compounds in antioxidant activity due to their ability to scavenge reactive oxygen species, including singlet oxygen, superoxide anions, and hydroxyl radicals. A strong correlation between TPC and antioxidant activity has been widely reported (Silva et al., 2022). Compared with *Coriolus versicolor* mushroom kombucha, which contains 330 mg GAE/L (Sknepnek et al., 2021), the mushroom kombucha samples in this study exhibited higher TPC values.

2.3 Total flavonoid content (TFC)

The total flavonoid content (TFC) of mushroom kombucha was assessed using the aluminum chloride colorimetric method and expressed as rutin equivalents (RUE). The results indicated that kombucha from Hed-Kor exhibited the highest flavonoid content (223.43±4.41 µg RUE/mL), followed by Hed Kai-Kiew (177.00±1.01 µg RUE/mL) and *C. cibarius* (157.71±2.22 µg RUE/mL). In comparison, non-filtered Wolffia kombucha was reported to contain 482 µg RUE/mL (Boonsupa & Insumran, 2025), highlighting the variability in flavonoid levels among different raw materials used for kombucha fermentation. The present findings support the notion that mushroom-based kombucha can serve as a valuable source of flavonoids, comparable to other plant-based kombucha beverages.

Table 1 Bioactive compounds of two wild mushroom species, *Russula* sp. (locally known as Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* kombucha during fermentation

Bioactive compounds	**Hed Kor**	**Hed Kai-Kiew**	***Cantharellus cibarius***
Antioxidant activity (mg α-TE/mL)	104.83±1.88^{c}	124.62±1.00^{b}	320.41±2.23^{a}
Total phenolic content (mg GAE/L)	328.29±2.49^{b}	441.52±3.74^{a}	223.29±2.07^{c}
Total flavonoid content (µg RUE/mL)	223.43±4.41^{a}	177.00±1.01^{b}	157.71±2.22^{c}

Remark: a, b, c values with various alphabet characters in the duplicate row are remarkably different (p<0.05).

3. Microbial population in kombucha fermented from different mushroom types

The microbial population in kombucha beverages fermented with *Russula* sp. (Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* was evaluated during the fermentation process. A commercial SCOBY (Symbiotic Culture of Bacteria and Yeast) was used as the inoculum. Microbial counts were determined on days 0 and 15 of fermentation.

3.1 Bacterial count

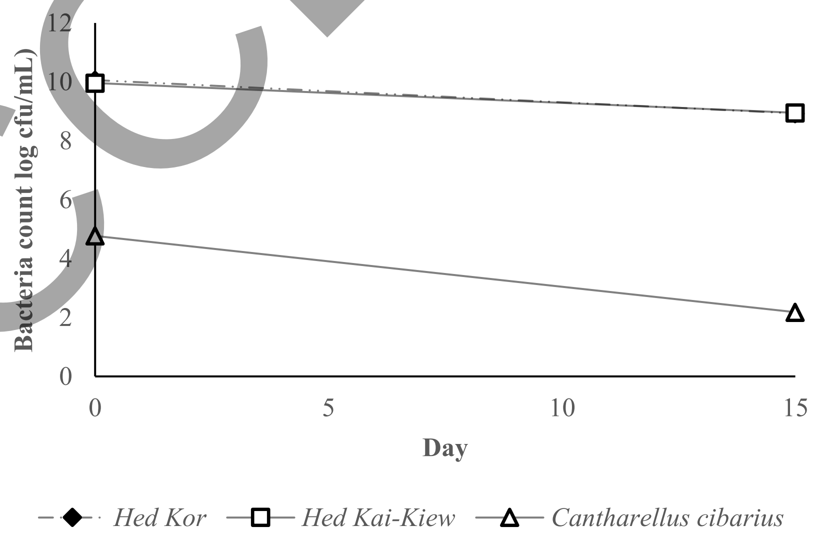
The bacterial population showed significant variation among the three mushroom-based kombucha samples. On day 0 the bacterial count in Hed Kor kombucha was 1.15×10^{10} CFU/mL, which decreased to 8.5×10^{8} CFU/mL by day 15. Hed Kai-Kiew kombucha had an initial bacterial count of 9.15×10^{9} CFU/mL, declining slightly to 9×10^{8} CFU/mL on day 15. Conversely, *Cantharellus cibarius* kombucha exhibited much lower bacterial counts throughout fermentation, starting at 5.95×10^{4} CFU/mL on day 0 and dropping further to 1.5×10^{2} CFU/mL on day 15 (Fig 3a). As Sknepnek et al. (2021) studied the *total number of microorganisms* during the 11-d fermentation of *Coriolus versicolor* and *Lentinus edodes* mushroom kombucha, it was found that the maximal number of acetic acid bacteria was reached on the 2nd day, in LE kombucha (7.56±0.01 log CFU) followed by a constant decrease afterwards.

3.2 Yeast and mold count

The yeast and mold populations increased substantially during fermentation, particularly in the Hed Kor and *Cantharellus cibarius* kombucha. Hed Kor kombucha showed an increase from 1.95×10^{9} CFU/mL on day 0 to 2.50×10^{14} CFU/mL on day 15. Hed Kai-Kiew kombucha increased from 2.81×10^{9} CFU/mL to 1×10^{12} CFU/mL. Remarkably, *Cantharellus cibarius* kombucha, which began with a relatively low yeast and mold count of 1.14×10^{5} CFU/mL, reached an extremely high level of 1.5×10^{7} CFU/mL by day 15 (Fig 3b), whereas Sknepnek et al. (2021), when they studied total number of microorganisms during the 11-d fermentation of *Coriolus versicolor* and *Lentinus edodes* mushroom kombucha, it was found that the maximal number of yeast was reached on the 2nd day, in LE kombucha (7.83±0.01 log CFU) followed by a constant decrease afterwards. According to Sittisart (2024) in an investigation of the total yeast of kombucha fermented with red tea leaves, pandan leaves, sucrose, hemp leaves, and milky mushroom flour during 21 d, the results showed that the total yeast increased during fermentation. The dynamic changes in microbial populations observed during fermentation are consistent with the known metabolic activities of SCOBY microorganisms. SCOBY plays a crucial role in metabolizing sugar and tea components into mildly acidic beverages rich in organic acids, polyphenols, and vitamins within 7–20 d of aerobic fermentation. Acetic acid bacteria and various yeast strains dominate the microbial community and contribute to both fermentation and inhibition of pathogenic contamination (Han et al., 2024). According to Han et al. (2024), microbial populations typically range between 10^6–10^8 CFU/mL after 9 d of fermentation. In this study, while bacterial counts decreased slightly or remained stable, yeast counts showed remarkable increases, especially in *Cantharellus cibarius* kombucha, which may be attributed to the mushroom's biochemical composition promoting yeast proliferation.

The observed reduction in bacterial counts alongside the disproportionate increase in yeast populations may be attributed to the biochemical composition of the mushroom substrates. Mushrooms differ in their levels of soluble carbohydrates, nitrogen, and secondary metabolites, which can selectively support or inhibit specific microbial groups. For instance, substrates rich in easily fermentable carbohydrates, such as those found in *Cantharellus cibarius*, may provide favorable conditions for yeast proliferation, thereby explaining the remarkable increase in yeast and mold counts despite low bacterial levels. Conversely, relatively low nitrogen availability and the possible presence of antimicrobial phenolic or terpenoid compounds in certain mushrooms could restrict bacterial growth, particularly acetic acid bacteria, which require balanced carbon and nitrogen sources. These compositional differences suggest that the selective enrichment of yeast over bacteria during fermentation is closely linked to mushroom substrate chemistry, which influences microbial succession and dominance throughout the fermentation process.

Compared with *Wolffia arrhiza* kombucha (Boonsupa & Insumran, 2025), this study's mushroom-based kombucha exhibited markedly higher antioxidant activity (320 vs. 18 mg/mL), although *Wolffia* kombucha contained substantially greater total phenolic and flavonoid contents (TPC 818 vs. 441 mg/L; TFC 482 vs. 223 µg/mL). Interestingly, acetic acid levels in *Wolffia* kombucha (2.35%) were almost threefold higher than in Hed Kai-Kiew kombucha (0.82%). Furthermore, while *Wolffia* kombucha showed a drastic decline in microbial counts by the end of fermentation, due to acid shock, this study recorded extremely high yeast and mold proliferation, which may be linked to the mushroom substrates or methodological factors. These comparisons emphasize that substrate type strongly influences both phytochemical enrichment and microbial dynamics in kombucha fermentation, and they underscore the importance of standardized microbial and chemical analyses to allow meaningful cross-study evaluations.



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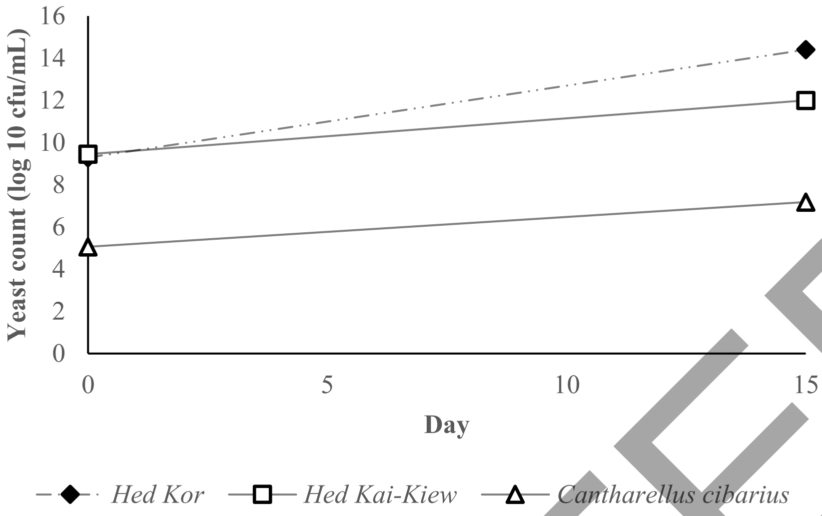

(b)

Fig 3. Microbial count of 2 wild mushroom species, *Russula* sp. (locally known as Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius* kombucha: a) Bacterial count and b) Yeast count

Conclusion

This study demonstrated the feasibility of producing kombucha beverages from Thai wild mushrooms, specifically *Russula* sp. (Hed Kor and Hed Kai-Kiew) and *Cantharellus cibarius*. Fermentation significantly influenced chemical and microbial properties, as well as the antioxidant potential of the beverages. Kombucha derived from *C. cibarius* exhibited the highest antioxidant activity, while *Russula* sp. showed greater total flavonoid content. Notably, Hed Kai-Kiew kombucha contained the highest total phenolic content and acetic acid concentration at the end of fermentation. These results suggest that mushroom substrates may enhance the bioactive properties of kombucha, highlighting their potential application in the development of functional beverages. The observed increases in phenolic and flavonoid compounds could be valuable for health-oriented products, aligning with consumer demand for natural antioxidants.

Nevertheless, several limitations must be addressed. The SCOBY inoculum was sourced commercially without microbial characterization, which may affect reproducibility. Moreover, factors such as the maturity stages of mushrooms and the uncontrolled particle size of the mushroom powder may have influenced the outcomes. Future studies should; (1) establish a laboratory-maintained mother culture to standardize the inoculum, (2) perform microbial quantification (e.g., plate counts) of starter cultures prior to fermentation, (3) apply standardized and traceable analytical methods, (4) control variables such as mushroom age and powder particle size, (5) ensure mushroom species authentication and toxicity verification, and (6) evaluate potential risks of microbial contamination due to extremely high yeast/mold growth. These improvements would enhance reproducibility and allow more reliable conclusions regarding the potential of mushrooms as alternative substrates for kombucha fermentation.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used Gemini (Google) to improve the clarity, readability, and language quality of the text. The final content was reviewed and edited by the authors, who take full responsibility for the accuracy and integrity of the publication.

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