



Production of Fermented Vinegar Drink From Hang Rice With Pineapple Peel Syrup

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Abstract: This study aimed to develop fermented vinegar beverages produced from Hang rice combined with pineapple peel syrup through traditional fermentation. The fermentation process was carried out under ambient conditions for 30 days using a traditional method to monitor changes in acetic acid bacteria and yeast populations, as well as acetic acid production. Consumer preference for the final product was also assessed. The microbial populations of both acetic acid bacteria and yeast showed a sharp increase during the first 14 days, reaching their peaks at 7.92 ± 0.24 log CFU/mL and 7.06 ± 0.28 log CFU/mL, respectively, after 30 days. The final vinegar exhibited $4.44 \pm 0.31\%$ acetic acid, a pH of 3.6, and showed no detectable alcohol. Three formulations were prepared by varying the ratio of Hang rice vinegar to pineapple peel syrup—Formula 1 (60:40), Formula 2 (50:50), and Formula 3 (40:60). Among them, Formula 3 demonstrated the highest DPPH radical scavenging activity (503.70 ± 24.42 μ g Trolox/mL) and the greatest total phenolic content (97.24 ± 4.36 μ gGAE/mL). Sensory analysis revealed comparable acceptability scores across all samples, ranging from “like slightly” to “like moderately”. These findings suggest that Hang rice vinegar supplemented with pineapple peel syrup represents a novel beverage with significant in vitro antioxidant capacity, adding value to germinated brown rice and promoting the utilization of pineapple peel waste.

Keywords: Vinegar; hang rice; pineapple peel syrup; acetic acid bacteria; antioxidant activity

1. Introduction

Vinegar is a naturally fermented product obtained through two sequential processes—alcoholic and acetic acid fermentation. Traditionally used as a sour condiment, preservative, and functional beverage, vinegar has recently gained attention for its potential health-promoting properties. Vinegar contains a wide range of bioactive compounds, including organic acids, phenolic acids, flavonoids, anthocyanins, melanoidins, tetramethylpyrazine, carotenoids, alkaloids, phytosterols, and vitamins, which have been associated with antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anti-obesity, antihypertensive, and cholesterol-lowering effects [1–4]. Among various types of vinegar, cereal-based vinegars produced from grains such as rice, barley, wheat, sorghum, and corn are particularly rich in bioactive compounds, including γ -oryzanol, γ -aminobutyric acid (GABA), and tetramethylpyrazine

[2]. Rice vinegar, widely consumed in Asian countries, especially China and Japan, has been extensively studied for its fermentation processes, bioactive composition, antioxidant and antibacterial activities, enzyme-inhibitory properties, and sensory characteristics [5–9]. Hang rice, a traditional product of northeastern Thailand, is derived from the local rice varieties Hom Mali 105 and glutinous RD6, through a process involving soaking, steaming, drying, and dehusking [10]. Germination enhances the nutritional profile of Hang rice by increasing the levels of γ -aminobutyric acid (GABA), phenolic compounds, γ -oryzanol, vitamin E, and other phytochemicals [11–14]. It is nutritionally rich in vitamin E, folate, pantothenic acid, B-group vitamins, niacin, and minerals such as magnesium, selenium, and calcium [15]. Pineapple peel, which accounts for approximately 35% of the total fruit weight, is a major by-product of the fruit processing industry and poses environmental concerns when discarded. Nevertheless, pineapple peel is rich in carbohydrates, dietary fiber, minerals, and phenolic antioxidants, making it a promising low-cost functional ingredient for sustainable food applications [16, 17].

Despite extensive studies on rice- and fruit-based vinegars, no previous research has reported the development of a fermented vinegar beverage derived from Hang rice. Moreover, the utilization of pineapple peel as a functional ingredient in vinegar beverages remains limited. This study, therefore, addresses this research gap by developing a novel functional fermented vinegar drink made from Hang rice and pineapple peel syrup. The objectives of this study were to optimize the formulation ratio between Hang rice vinegar and pineapple peel syrup, investigate the population dynamics of yeast and acetic acid bacteria during fermentation, and evaluate the total phenolic content, antioxidant activity, and sensory acceptability of the developed product.

2. Materials and Methods

2.1 Materials

The Hang rice variety used in this study was Khao Dawk Mali 105, obtained from the Ban Samran Suk Hang Rice Homemakers Community Enterprise, Nong Bua Lam Phu Province, Thailand. Pineapple peels were collected from a local fruit market in Chokchai, Nakhon Ratchasima, and *Loog-pang* (a traditional starter culture) was purchased from a Thai SME located in Mueang District, Maha Sarakham Province.

2.1.1 Production of Hang rice vinegar

Hang rice was washed, soaked overnight in water, drained, and steamed for 30 min. After cooling to room temperature, the steamed rice was inoculated with 1.0% (w/w) *Loog-pang* and transferred to a 3 L glass jar for saccharification at 30–35 °C for 10 days. The liquid fraction obtained after saccharification was filtered and adjusted to 18 °Brix. Alcoholic fermentation was initiated by adding 200 mL of sterile water. The glass jar was loosely covered with sterile cheesecloth and a lid to prevent contamination while allowing limited gas exchange. Fermentation was carried out at ambient room temperature (30 ± 2 °C) for 4 days, resulting in a total fermentation period of 14 days. After alcoholic fermentation, the rice wine was pasteurized at 60 °C for 15 min, and the ethanol concentration was standardized to 8% (v/v) using distilled water to ensure consistent initial conditions for acetic acid fermentation. The acetic acid fermentation was then conducted under aerobic conditions for 16 days, bringing the total fermentation period to 30 days. Samples were collected at defined intervals to determine ethanol content, total acidity, pH, and microbial populations, and to evaluate the fermentation kinetics throughout the process. The growth of acetic acid bacteria (AAB) and yeasts was monitored over the 30-day fermentation period using the spread plate method to determine viable cell counts. Briefly, 100 μ L aliquots from appropriate serial dilutions were spread onto Glucose Yeast Calcium Carbonate (GYC) agar for enumeration of AAB and onto yeast extract peptone dextrose (YEPD) agar for yeast counts. All samples were plated in triplicate and incubated at 30 °C for 24–48 h for AAB and 48 h for yeasts.

2.1.2 Analysis of physicochemical properties

The vinegar samples were analyzed for pH, total soluble solids (TSS), alcohol content, and acetic acid concentration. All samples were analyzed in triplicate. pH values were determined using a pH meter (AB15, Hampton, USA), while TSS was measured with a refractometer (RHB-32ATC, Yago Technology, China). Alcohol content was assessed using an ebulliometer (Alla France, France) by measuring the difference in

boiling points between water and the sample solution. The total acidity of the vinegar samples was determined by titration using AOAC Official Method 930.35 [18]. A 10 mL aliquot of the sample was diluted with distilled water and titrated against a standardized 1N NaOH solution using phenolphthalein as an indicator. The total acidity was calculated and expressed as grams of acetic acid per 100 mL of sample (% w/v), calculated as follows (Equation 1):

$$\text{Acetic acid (g/100 mL)} = \frac{C \times V \times MW \times 100}{1,000 \times 10} \quad (1)$$

Where C is the NaOH concentration, V is the titration volume, and MW is the molar mass of acetic acid.

2.2 Production of fermented vinegar drink from Hang rice with pineapple peel syrup

2.2.1 Preparation of pineapple peel syrup

Pineapple peels were washed, trimmed, and cut into thin pieces. The peels (250 g) were combined with brown sugar (200 g), two cinnamon sticks (5 g), lemon juice (20 mL), and water (250 mL). The mixture was boiled, then simmered for 20 minutes while gently crushing the fruit until thickened. The syrup was filtered through muslin cloth, cooled, and stored at 4 ± 1 °C.

2.2.2 Formulation of Fermented Vinegar Drink

Three formulations were prepared by blending fermented Hang rice vinegar with pineapple peel syrup at ratios of 60:40 (Formula 1), 50:50 (Formula 2), and 40:60 (Formula 3). The mixtures were filtered, bottled (250 mL glass bottles), sealed, and stored at 4 ± 1 °C for antioxidant and sensory analyses.

2.3 Sensory evaluation of fermented Hang rice vinegar drink with pineapple peel syrup

The fermented Hang rice vinegar drink with pineapple peel syrup was evaluated on a 9-point hedonic scale. Thirty untrained panelists evaluated six attributes: appearance, color, aroma, taste, and overall acceptability. On this scale, a value of 9 represented 'like extremely,' 5 represented 'neither like nor dislike,' and 1 represented 'dislike extremely.'

2.4 Antioxidant activity analyses of the fermented Hang Rice vinegar drink with pineapple peel syrup

2.4.1 Determination of Total Phenolic Content

The total phenolic content of the fermented Hang rice vinegar drink with pineapple peel syrup was determined using the Folin-Ciocalteu reagent. Briefly, 50 µL of each sample was diluted with distilled water, then mixed with 100 µL of Folin-Ciocalteu reagent and 2 mL of 2% Na₂CO₃ solution. After a 30-minute incubation at room temperature, the absorbance was measured at 750 nm using a UV-Vis spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific, USA). The results were expressed as micrograms of gallic acid equivalents per 1 mL of sample (µg GAE/mL).

2.4.2 Determination of DPPH Radical Scavenging Activity

The antioxidant activity of the fermented Hang rice vinegar drink with pineapple peel syrup was evaluated using the DPPH radical assay, where the DPPH radical served as a stable radical. In brief, 50 µL of each sample was added to 1.950 mL of a 40 mg/L DPPH radical solution prepared in ethanol. The mixture was then incubated for 30 minutes at room temperature in the dark. After the incubation period, the absorbance was measured at 515 nm using a UV-Vis spectrophotometer, and the scavenging activity was calculated using Equation 2.

$$\text{scavenging activity (\%)} = ((A_{\text{DPPH}} - A_{\text{sample}})/A_{\text{DPPH}}) \quad (2)$$

where A_{DPPH} and A_{sample} denote the absorbance of the control and sample, respectively.

The antioxidant activity was calculated from a standard curve of Trolox, and the results were expressed as milligrams of Trolox equivalents per milliliter of sample µg TE/mL).

2.5 Microbiological Safety Assessment

The microbiological safety of the fermented Hang rice vinegar drink was evaluated according to the BAM (Bacteriological Analytical Manual) standard methods. Samples from the optimal formulation were analyzed for Total Plate Count (TPC), Coliforms, yeasts and molds, and the presence of pathogenic microorganisms (*Escherichia coli* and *Salmonella* spp.). Ten milliliters of each sample were serially diluted in 0.1% peptone water. TPC and yeast/mold counts were determined using Plate Count Agar (PCA) and Potato Dextrose Agar (PDA), respectively, after incubation at 35°C for 48 hours and 25°C for 5 days. The results were expressed as colony-forming units per milliliter (CFU/mL). All tests were performed in triplicate.

2.6 Statistical Analysis

All analyses were carried out in triplicate, and results were expressed as mean \pm standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA). Statistical differences among treatments were determined by Duncan's multiple range test at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1 Changes in chemical and microbiological parameters during fermentation of Hang rice vinegar

The traditional static fermentation of Hang rice vinegar for 30 days is illustrated in Figure 1. Cooked Hang rice can be fermented into Khao Mak within 24 hours. After the first day of fermentation, the sample developed a pale yellowish-white color and a pleasant aroma with mild alcoholic notes, accompanied by a small amount of clear exudate (*Nam-toy*). Subsequently, during fermentation, the alcohol content slowly increased until day 21. At the end of fermentation (30 days), the vinegar exhibited a clear to light yellow appearance and a slightly pungent smell, attributed to the presence of acetic acid (Figure 1).

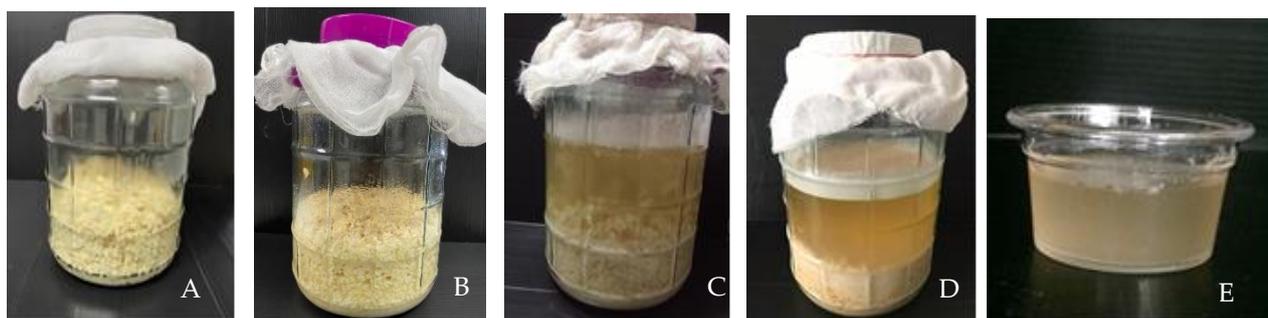


Figure 1. Appearance of Hang rice vinegar during traditional static fermentation; (A) beginning of fermentation; (B) 10 days (before alcoholic fermentation); (C) 21 days; (D) 30 days; (E) the final Hang rice vinegar product obtained after 30 days of fermentation.

The dynamic changes in microbial populations, ethanol and acetic acid production, and pH during fermentation are shown in Figure 2. Both yeast and acetic acid bacteria (AAB) populations increased markedly within the first 14 days of fermentation. Maximum cell densities of AAB (7.92 ± 0.24 log CFU/mL) and yeasts (7.06 ± 0.28 log CFU/mL) were observed at day 30, indicating sustained microbial activity throughout the fermentation process. Ethanol concentration increased significantly during the early fermentation stage, reaching a maximum of $9.5 \pm 0.4\%$ (v/v) after 14 days, reflecting the efficient conversion of fermentable sugars into ethanol by yeast cells. The relatively high ethanol yield during this period can be attributed to the starch-rich nature of Hang rice, combined with continuous saccharification within the fermentation system. Given the high starch content of Hang rice, saccharification represents a critical prerequisite for effective alcoholic fermentation [19, 20]. This process generates fermentable sugars, including glucose and oligosaccharides, through the action of α -amylase and related enzymes, which are subsequently metabolized by yeasts into ethanol and later oxidized into acetic acid during vinegar fermentation [21, 22]. Traditional starter cultures such as *Loog-pang* harbor diverse fungal and yeast communities capable of producing amylolytic enzymes that hydrolyze complex starch into fermentable sugars, thereby ensuring a sustained supply of substrates to support yeast metabolism during rice-based alcoholic fermentation. This process is mediated by the complex microbial consortium present in *Loog-pang*, which includes molds (e.g., *Aspergillus*, *Amylomyces*, *Rhizopus*, and

Mucor spp.), yeasts (e.g., *Saccharomyces cerevisiae* and *Candida* spp.), and associated bacteria that collectively secrete amyolytic enzymes, facilitating starch degradation and sustained alcohol formation [23–26]. Following the alcoholic fermentation stage, ethanol concentration declined significantly to $6.2 \pm 0.3\%$ (v/v) and became undetectable by day 30. In contrast, acetic acid concentration increased sharply, reaching $4.44 \pm 0.31\%$ (w/v) by day 21, accompanied by a decrease in pH from 6.5 to 3.6. These results comply with the Codex Alimentarius standard for fermented vinegar (CXS 162-1987), which specifies a minimum acetic acid content of 4% (w/v), residual ethanol levels below 0.5% (v/v), and a typical pH range of 2.5–3.5 [27]. The inverse relationship between ethanol depletion and acetic acid accumulation reflects AAB's metabolic activity, which oxidizes ethanol to acetic acid under aerobic conditions. Vinegar production involves two distinct yet interrelated fermentation stages. During the initial alcoholic fermentation, yeasts—predominantly *Saccharomyces cerevisiae*—convert fermentable sugars into ethanol [28, 29]. In addition to ethanol production, yeast metabolism releases amino acids, vitamins, and autolysis-derived compounds that stimulate the growth and metabolic activity of AAB [30]. Subsequently, acetic acid fermentation is driven mainly by *Acetobacter* spp., which oxidize ethanol to acetic acid via the sequential action of membrane-bound alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes [29, 31]. In this study, spontaneous fermentation was employed to exploit the indigenous microbiota naturally associated with Hang rice and pineapple peels, thereby promoting microbial interactions and metabolic synergy. This approach results in a more complex metabolic profile and distinctive sensory characteristics compared with pure-culture inoculation [32–34]. Such microbial interactions are often associated with enhanced production of bioactive compounds and increased antioxidant potential [19]. Moreover, spontaneous fermentation aligns with traditional artisanal vinegar production practices, where diverse microbial communities play a crucial role in developing unique functional and sensory attributes [34]. From a practical perspective, this strategy also supports a cost-effective and sustainable production model by reducing reliance on commercial starter cultures, thereby improving accessibility for small-scale producers and local communities [35, 36].

Colony morphology of isolated yeast and AAB from Hang rice vinegar after 30 days of fermentation is shown in Figure 3. The yeast isolates exhibited white, powdery, rough colonies with raised elevation and undulated margins, which are commonly associated with yeast involved in alcoholic fermentation, as reported in previous studies [37–39]. In the literature, various yeast species, including *Saccharomycopsis fibuligera*, *Pichia* spp., *Issatchenkia* spp., *Candida* spp., and *Torulasporea* spp., have been reported in traditional rice-based starters such as Loog-pang and Medombae [40, 41]. These references are provided for comparative context, as species-level identification was not performed in the present study. AAB colonies exhibited small, creamy-white circular forms with convex elevation and clear zones around colonies, which are characteristic features of acetic acid-producing bacteria. Consistently, *Acetobacter* species have been reported to form beige, circular colonies on glucose, yeast extract, and peptone agar [42]. Microbial identification in this study was based on culture-dependent methods, including plate counts and colony morphology, which provide presumptive identification of yeast and acetic acid bacteria. These approaches are commonly applied in vinegar fermentation studies and are suitable for evaluating overall microbial population trends during fermentation. However, they do not provide precise taxonomic resolution. Therefore, the observed microbial dynamics should be interpreted as indicative population trends. Future studies employing molecular identification techniques, such as 16S rRNA gene or ITS sequencing, would further strengthen microbial characterization.

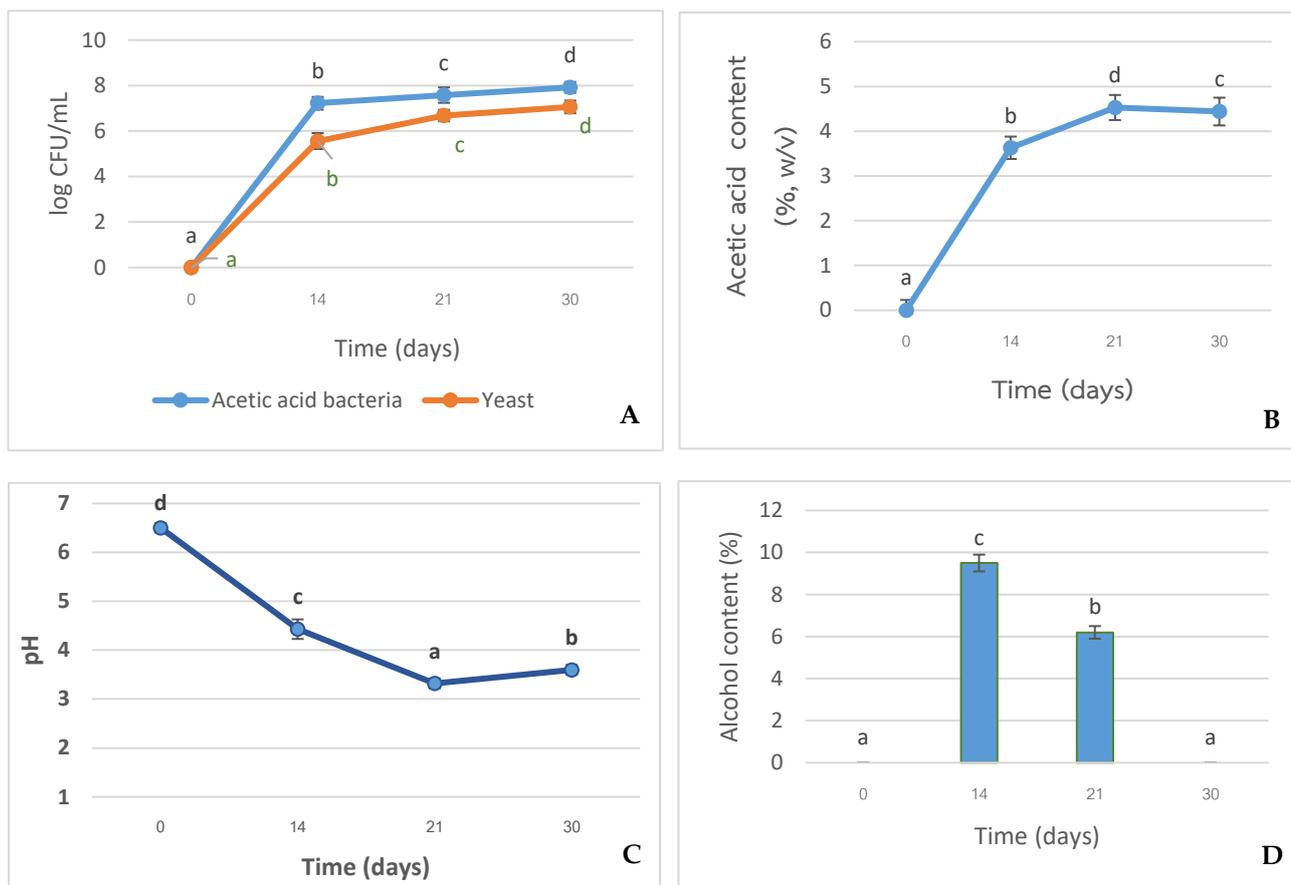


Figure 2. Dynamic changes in microbial populations (AAB and Yeast) (A), acetic acid content (% w/v) (B), pH (C), and alcohol content (% v/v) (D), during the 30-day traditional static fermentation of Hang rice vinegar. Each data point represents the mean \pm standard deviation (n=3). Significant differences ($p < 0.05$). Different letters show significant differences ($p \leq 0.05$) among vinegar samples during the fermentation period.



Figure 3. Colony morphology of isolated yeast from Hang rice vinegar on YEPD agar (B), isolated acetic acid bacteria from Hang rice vinegar showing formation of a halo zone surrounding their colonies grown on GYC agar.

3.3 Production of fermented Hang rice vinegar drink with pineapple peel syrup

The pineapple peel syrup exhibited an amber-brown, viscous, and slightly turbid appearance with a distinctive fruity, sweet, and spicy aroma (Figure 4). The syrup had total soluble solids of 16°Brix and a pH of 5.4. The final vinegar drink formulations (Figure 5) showed distinct color variations: Formulas 1 and 2 displayed an amber-yellow hue with slight turbidity, while Formula 3 showed a bronze-orange tone.



Figure 4. Preparation process of pineapple peel syrup (A) and the final pineapple peel syrup product (B).

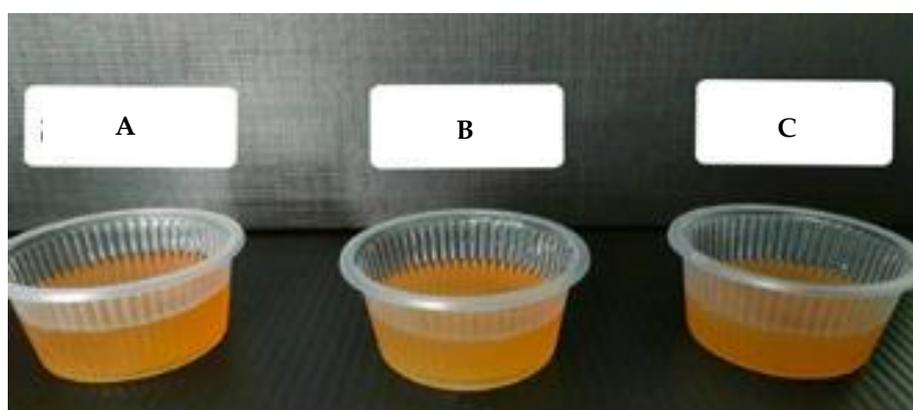


Figure 5. The appearance of three fermented Hang rice vinegar drink formulations, prepared with Hang rice vinegar and pineapple peel syrup in *different ratios*: (A) formula 1(60:40); (B) formula 2 (50:50), and formula 3 (40:60).

3.3 Sensory evaluation of Hang rice vinegar drink with pineapple peel syrup

The sensory characteristics of the three Hang rice vinegar drink formulations are presented in Figure 6. All three samples achieved moderate consumer acceptability, with mean scores ranging from 5.4 to 7.0. No significant differences were observed among formulations in appearance, with ratings classified as “like slightly” (6.43 ± 1.29 – 6.63 ± 1.29). In terms of color, Formula 1 obtained the highest score (“like moderately”; 6.83 ± 1.26), comparable to Formula 2, while Formula 3 received a lower rating (6.10 ± 1.26), possibly due to its darker, brownish hue compared with the brighter tone of Formula 1. Aroma scores also showed no significant difference among the samples, ranging between “neither like nor dislike” and “like slightly” (5.40 ± 1.88 – 6.10 ± 1.88). Similarly, taste scores did not differ significantly, ranging within the “like slightly” category (6.10 ± 1.52 – 6.70 ± 1.52). The overall acceptability of all three vinegar drinks did not differ significantly either, ranging from “like slightly” to “like moderately” (6.76 ± 1.56 – 7.00 ± 2.34). These findings align with those of Saithong et al. [43], who reported overall acceptability scores of 7.05-7.18 for rice vinegars produced from various Thai rice varieties, including Khao Dawk Mali 105, Sangyod, and Hom Nil. Vinegar coloration depends primarily on raw material pigments and the chemical transformations that occur during fermentation and storage, including the Maillard reaction and enzymatic browning [44]. Likewise, the aroma and flavor profiles of vinegar are shaped by multiple factors, including substrate composition, fermentation conditions, and the presence of volatile organic compounds. Earlier studies have identified acetic acid and other short-chain organic acids (e.g., propionic, tartaric, and butyric acids), as well as esters and alcohols, as the major contributors to vinegar’s characteristic aroma and taste [45]. Acetic acid imparts the characteristic sourness, pungent aroma, and sharp flavor typical of vinegar products [46–48]. Esters, produced during alcoholic and acetic acid fermentation or during aging, provide pleasant floral and fruity notes typical of fruit vinegars [49, 50].

Alcohols, mainly generated during alcoholic fermentation, add additional fruity, fatty, and fragrant characteristics [51]. Therefore, these findings suggest that Hang rice vinegar blended with pineapple peel syrup can be developed into a novel functional beverage that combines favorable sensory qualities with potential nutritional and health benefits.

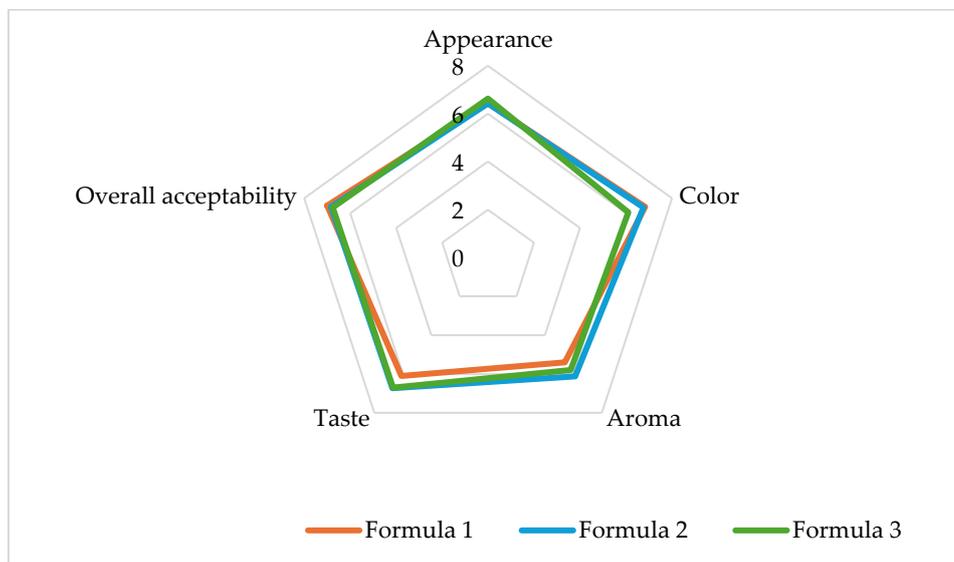


Figure 6. Sensory evaluation radar chart of three Hang rice vinegar drinks with pineapple peel syrup formulation.

3.4 Antioxidant Activity of Hang rice vinegar drink with pineapple peel syrup

3.4.1 Scavenging activity towards DPPH free radicals

The antioxidant capacity of three Hang rice vinegar drink formulations supplemented with pineapple peel syrup is illustrated in Figure 7. Among the tested samples, Formula 3 exhibited the highest scavenging activity ($503.70 \pm 24.42 \mu\text{g Trolox/mL}$), followed by Formula 1 ($465.58 \pm 8.90 \mu\text{g Trolox/mL}$) and Formula 2 ($413.64 \pm 21.78 \mu\text{g Trolox/mL}$). The superior antioxidant performance of Formula 3 is likely attributed to the higher contribution of Hang rice vinegar, which is rich in bioactive compounds such as phenolics, flavonoids, γ -oryzanol, tocopherols, and tocotrienols [52]. These findings are consistent with Suwannawong et al. [53], who reported Hang rice extract showed DPPH radical scavenging activity with an IC_{50} of $594.625 \mu\text{g/mL}$, while Phattayakorn et al. [54] observed 73.6% DPPH inhibition in germinated Hang rice (jasmine KDML105). Moreover, rice vinegars such as Hom Nil, Sangyod, and Riceberry have been shown to exhibit antioxidant activity ranging from 49.27% to 74.95% [43, 55]. Meanwhile, pineapple peel contains high levels of natural antioxidants, including phenolic compounds, flavonoids, and vitamin C [56]. Lourenço et al. [57] reported antioxidant activity of $91.79 \pm 1.98 \mu\text{mol Trolox/g}$ dry extract for pineapple peel using the DPPH assay. In addition to antioxidant activity, pineapple peel extracts exhibit anti-inflammatory, antiviral, antiparasitic, antifungal [58], and antimicrobial effects against common foodborne pathogens [59].

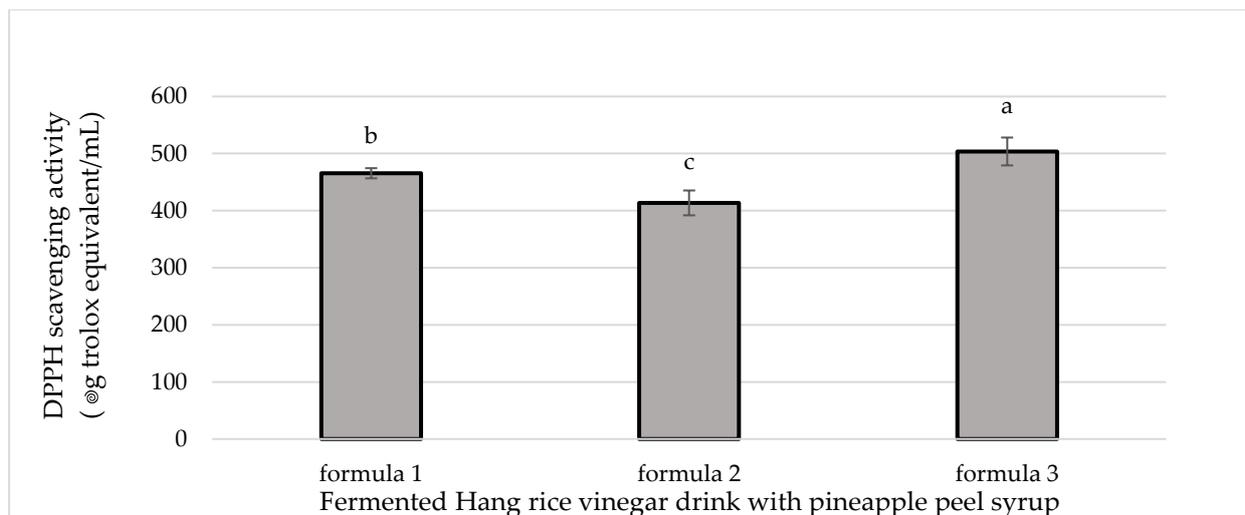


Figure 7. DPPH radical scavenging activity of Hang rice vinegar drink with pineapple peel syrup. Different letters show significant differences ($p \leq 0.05$) among fermented vinegar drink formulations.

3.4.2 Total phenolic contents of Hang rice vinegar drink with pineapple peel syrup

As shown in Figure 8, Formula 3 recorded the highest total phenolic content ($97.24 \pm 4.36 \mu\text{g GAE/mL}$), followed by Formula 1 ($84 \pm 3.15 \mu\text{g GAE/mL}$) and Formula 2 ($78.16 \pm 2.59 \mu\text{g GAE/mL}$). These variations may result from differences in the proportions of Hang rice vinegar and pineapple peel syrup. Phenolic compounds are abundant in both rice and pineapple peels and function as major antioxidants [60, 61]. In rice, phenolic acids, anthocyanins, and proanthocyanidins represent the predominant bioactive constituents [62]. Specifically, ferulic, p-coumaric, sinapic, and vanillic acids are highly concentrated in whole grains and germinated brown rice [63]. These are also the primary bioactive components in Hang rice, as their levels are further enriched by the unique germination and hydrothermal treatment processes (soaking, steaming, drying, and dehusking). These traditional preparation stages significantly enhance the bioavailability of phenolics, γ -oryzanol, vitamin E, and GABA [64–66]. Furthermore, during vinegar fermentation, these compounds undergo microbial biotransformations catalyzed by yeast and acetic acid bacteria (AAB), which may enhance their antioxidant potential and health-related properties [24, 33, 67]. Consequently, these transformed compounds exhibit diverse biological activities, including antioxidant [68], antimutagenic, anticancer, anti-allergic, and cholesterol-lowering effects [69–71]. Previous reports have indicated that Hang rice contains higher phenolic content than brown rice, reaching up to 17.857 mg GAE/g [60], and germinated Hang rice (KDML105) as high as 437.16 mg GAE/g [72]. Pineapple peel also contains diverse phenolic constituents, including flavonoids and acids such as gallic, catechin, epicatechin, ferulic, salicylic, myricetin, trans-cinnamic, tannic, and p-coumaric acids [67, 68]. Campos et al. [73] and Lourenço et al. [57] confirmed that pineapple peel extract possesses higher phenolic levels than stem extract, with $11.10 \pm 0.01 \text{ mg GAE/g}$ dry extract. During acetic acid fermentation, phenolic composition may change due to oxidation and hydrolysis reactions [74–77]. Previous reports confirmed that rice vinegar generally exhibits substantial phenolic content and antioxidant capacity. Pokkakaw and Manangsattit [78] found that Riceberry and Kao Luem Pua vinegars contained 17.5 ± 0.27 and $9.75 \pm 0.33 \text{ mg GAE/100 mL}$, respectively, while Taweekasemsombut et al. [79] reported $133.68 \text{ mg GAE/100 mL}$ for Black glutinous rice vinegar. Similarly, Saithong et al. [43] observed $428.47 \pm 4.04 \text{ GAE/mL}$ in Hom Nil vinegar, and another study [80] reported $50.7 \pm 2.8 \text{ mg GAE/mL}$ for germinated brown rice vinegar. These findings collectively confirm that Hang rice vinegar combined with pineapple peel syrup exhibits strong antioxidant potential, primarily due to the synergistic effects of the phenolic compounds and other bioactive constituents present in both substrates. Furthermore, the antioxidant capacity of the developed Hang rice vinegar drink was within the reported range for various commercial fruit vinegars, such as pineapple and rosehip. Similarly, the total phenolic content observed in this study ($97.24 \pm 4.36 \mu\text{g GAE/mL}$) aligned with the values reported for other commercial fruit vinegar beverages, which typically range from

96.21 to 4,441.25 mg GAE/L [81]. This comparison confirms that the developed formulation is competitive with established commercial products in terms of functional properties.

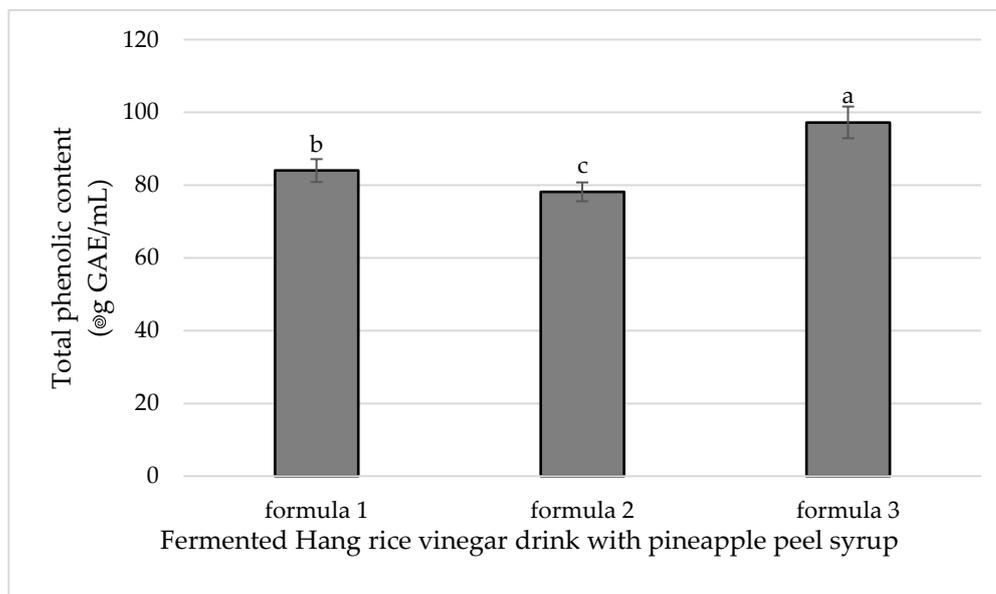


Figure 8. The total phenolic content of Hang rice vinegar drink with pineapple peel syrup. Different letters show significant differences ($p \leq 0.05$) among fermented vinegar drink formulations.

In addition, the microbiological quality of the Hang rice vinegar drink was evaluated using Total Plate Count (TPC) and coliform analysis. The results showed that the total viable count of the fermented vinegar drink was below the countable range (< 30 CFU/mL), while the coliform count was below 1.1 MPN/100 mL. No pathogenic microorganisms were detected under the analytical conditions employed, as shown in Table 1. These microbiological parameters were within the acceptable limits specified by the Thai Community Product Standard for Vinegar Drink (TIS No. 1287/2550), indicating that the Hang rice vinegar drink met the microbiological quality requirements for fermented beverages.

Table 1. Microbiological safety assessment of Hang rice vinegar drink supplemented with pineapple peel syrup.

Microorganisms	Testing results
Total viable count, CFU/mL	Not detected (< 30 CFU/mL)
Coliforms, MPN/100 mL	< 1.1
Yeast and Mold, CFU/mL	Not detected
<i>Escherichia coli</i>	Not detected
<i>Salmonella</i> spp.	Not detected

4. Conclusions

This study demonstrated the successful development of a novel functional vinegar beverage from Hang rice combined with pineapple peel syrup. During 30 days of fermentation, the populations of acetic acid bacteria and yeast reached 7.92 log CFU/mL and 7.06 log CFU/mL, respectively, while the final vinegar contained 4.44% acetic acid, exhibited a pH of 3.6, and had no detectable alcohol. Among the three tested formulations, Formula 3 (Hang rice vinegar to pineapple peel syrup ratio of 40:60) was identified as the most suitable. This formulation exhibited the highest antioxidant activity (503.70 ± 24.42 µg Trolox/mL) and the greatest total phenolic content (97.24 ± 4.36 GAE/mL), along with a favorable sensory score of 6.70 ± 1.52 for overall acceptability. These results suggest that Formula 3 is the optimal formulation for a functional Hang rice vinegar drink enriched with pineapple peel syrup, offering promising in vitro antioxidant properties and demonstrating high consumer preference. Although this work demonstrates the successful development of

the vinegar drink, the findings are considered preliminary regarding consumer safety. Further studies are required to evaluate the product's shelf-life stability over an extended storage period.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

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