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Nutritional compositions, microbial quality, bioactivities, and volatile compounds of a novel vinegar from wild edible mushroom, *Russula delica* Fr

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Abstract

Vinegar is commonly utilized in cooking and food preparation as a flavoring, preservative, and condiment. It can be made from various sources, including fruits, grains, and vegetables. This study produced vinegar from a wild edible mushroom, *Russula delica* Fr., using microwave-assisted enzymatic hydrolysis extraction. The nutritional composition, bioactivities, microbial quality, and volatile compounds were analyzed in the production process and final vinegar product. Sugar syrup as total soluble solids (TSS) and total phenolic content (TPC) were extracted from mushroom powder using commercial enzymes and yielded 5.60 ± 0.10°Brix and 7.01 ± 0.06 mg GAE/g substrate, respectively. The extracted syrup was rich in amino acids such as aspartic and glutamic acid, with glucose as the main type of sugar. Maximum alcohol content at 10.95 ± 0.21% (w/v) with 1.28 ± 0.23 mg GAE/mL TPC was obtained from *Saccharomyces cerevisiae* fermentation after 21 days, while highest acetic acid was obtained at 5.60 ± 0.42% w/v with 1.87 ± 0.14 mg GAE/mL of TPC content and 74.85 ± 1.24% of DPPH radical scavenging activity after surface fermentation using *Acetobacter aceti* TISTR 354. Thirteen volatile compounds, including acids, alcohols, and aldehydes, were found in the wild edible mushroom vinegar, contributing to the unique aroma of the product. This study presented the first report on the analysis of vinegar from a wild edible mushroom, *R. delica* Fr. which showed high nutritional value, antioxidant activity and volatile compounds, with the potential for future commercial production.

Keywords Vinegar fermentation, Wild edible mushroom, *Russula delica* Fr, Microwave-assisted enzymatic hydrolysis, Heavy metals

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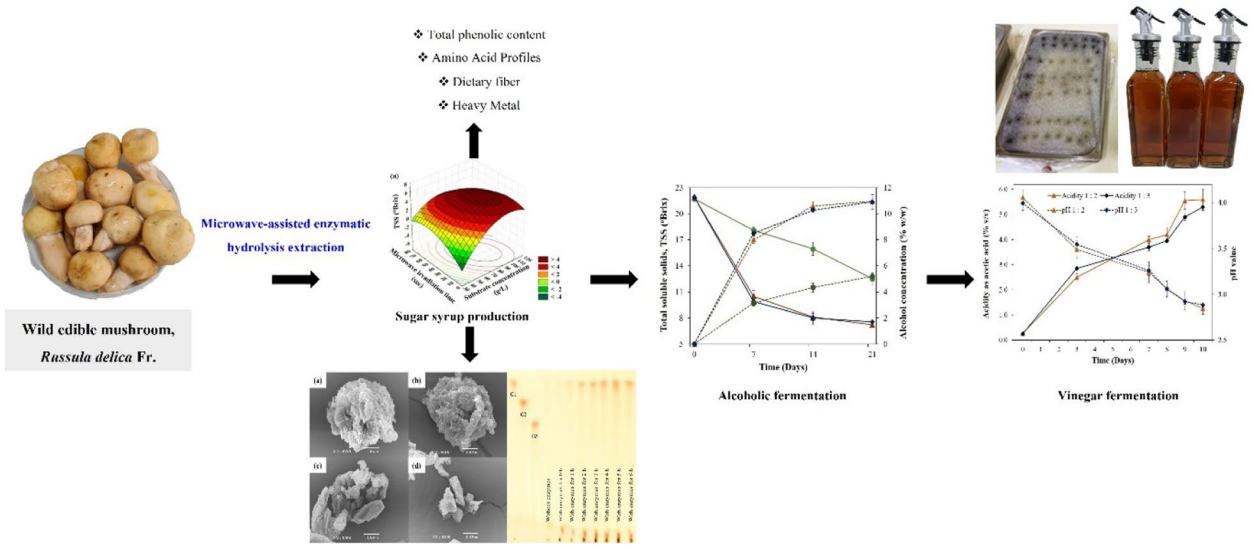
Graphical Abstract



Introduction

One of the most popular acidic condiments is vinegar, mainly acetic acid, obtained from the two-step fermentation process of alcoholic fermentation followed by acetic acid fermentation (Dias et al. 2016; Bae et al. 2022; Zhang et al. 2020). Alcoholic fermentation is the anaerobic conversion of sugars to ethanol by yeast strains such as *Saccharomyces cerevisiae* (Majumder et al. 2022), while acetic acid fermentation is the aerobic oxidation of ethanol to acetic acid by acetic acid bacteria such as *Acetobacter aceti* (Molelekoa et al. 2018; Sangngern et al. 2020). Historically, vinegar has been utilised as a food flavouring, medicine, preservative and cleaning agent (Zhang et al. 2020). Vinegar is traditionally fermented from fruits, grains, vegetables and rice (Li et al. 2014; Lomthong & Saithong 2019; Molelekoa et al. 2018; Sangngern et al. 2020; Zhang et al. 2020). However, to the best of our knowledge, no reports have been published on vinegar generation from the wild edible mushroom, *R. delica* Fr., as a new nutritional bioactive compound.

The edible ectomycorrhizal wild mushroom *R. delica* Fr., also known as milk-white brittlegill and classified under the family Russulaceae is a new source for medical, cosmetic, and food applications (Khatua et al. 2013; Oscar et al. 2019). Extracts of *Russula* spp. were found to contain lectins with antioxidant and antibacterial properties (Kostić et al. 2020; Yaltirak et al. 2009) that potently inhibited the proliferation of cancer cells and HIV-1 reverse transcriptase activity (Zhao et al. 2010). Consuming this mushroom promotes health benefits; however, utility as a health supplement is not well recognised. According to Oscar et al. (2019), *Russula* spp. contained 17 amino acids, both essential and non-essential, and could be considered a potential health food as well as a source of ingredients for the food industry.

Microwave-assisted enzymatic hydrolysis combines microwave irradiation that generates heat and enzymatic degradation to hydrolyse the substrate, thereby increasing hydrolysis efficiency to extract sugar syrup and bioactive compounds (Tsubaki et al. 2013). Thongpoem et al. (2021) found that the application of microwave-assisted starch-degrading enzyme hydrolysis for sugar syrup production from unripe banana flour increased hydrolysis yield and reduced hydrolysis time for incubation.

Heavy metals in vinegar have been widely studied because they can come from raw materials and manufacturing processes and can be harmful at high concentrations (Salman & Shamar 2013; Topdas 2023). Heavy metal analysis in the novel substrate or manufacturing processes were required to create a safe novel product.

Therefore, here, vinegar production from a wild edible mushroom, *R. delica* Fr., was developed by microwave-assisted enzymatic hydrolysis extraction, and analysis of the nutritional composition, bioactivities, microbial quality, and volatile compounds for further applications was carried out.

Materials and methods

Mushroom and enzyme preparation

Basidiocarps of *R. delica* Fr. were obtained from Talaad Thai Market, Pathum Thani, Thailand and identified by morphological features compared to the standard classification (Kaewgrajang et al. 2020). The mushrooms were rinsed twice in tap water, dried at 50 °C in a hot air oven for 12 h, powdered using a multipurpose grinder (GM-800S1, China), filtered through 60-mesh and kept under dry conditions. The chemical composition of *R. delica* Fr. was analyzed for protein, starch, fat, hemicellulose, cellulose, lignin, and fiber contents using AOAC methods (Helrich 1990), as reported by Noree et al. (2021) and Chorum et al. (2022).

Commercial enzymes purchased from the Reach Biotechnology Co., Ltd. (Thailand) included liquid xylanase (iKnowZyme® XL) and powdered cellulase (iKnowZyme® Cellulase). The enzymes were kept at -20 °C until required (Chaiyaso et al. 2019).

Microorganism and inoculum preparation

Alcoholic fermentation yeasts, *Saccharomyces cerevisiae* sub. *burgundy* and *S. cerevisiae* sub. *montache* were obtained from the Institute of Food Research and Product Development (IFRPD), Kasetsart University, Thailand. The Saccharomyces strains were grown in yeast malt (YM) agar slants (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone and 15 g/L agar supplemented with 10 g/L glucose). Each tube of yeast strain in the agar slant was mixed in the inoculum medium (100 mL) containing diluted 1:2 mushroom hydrolysis with distilled water (adjusted to 22°Brix by sucrose) supplemented with diammonium phosphate (DAP) at 1.2 g/L, with the pH adjusted to 4.0 using citric acid. After incubation at 30 °C for 16 h, 10% (v/v) was used as inoculum for mushroom wine fermentation (Sangngern et al. 2020).

The acetic acid bacterium, *A. aceti* TISTR 354, was obtained from the IFRPD, Kasetsart University, Thailand. The inoculum was prepared by cultivating the bacterial strain in liquid medium containing 7.0 g mushroom powder, 76.0 mL distilled water and 7.0 mL of 95% ethanol, modified from Lomthong and Saithong (2019) and Sangngern et al. (2020). The culture was incubated at 30 °C without shaking for 4 days and used as inoculum at 10% (v/v), approximately 10^8 CFU/mL, on a De Man, Rogosa & Sharpe (MRS) agar plate (Saithong et al. 2017).

Microwave-assisted enzymatic hydrolysis extraction

Response surface methodology (RSM) with central composite design (CCD) was applied to evaluate the production of sugar syrup containing phenolic compounds from the wild edible mushroom *R. delica* Fr. The hydrolysis reaction was performed in 250 mL Erlenmeyer flasks containing 49 mL of cellulase enzyme solution (5% w/v in distilled water) and 1.0 mL of liquid xylanase. The mushroom concentration (X_1) was added to the reaction following five levels of central composite design (CCD) including 0, +1, -1, -α and +α, as shown in Table 1. The reaction pH was adjusted to 5.5 by 5.0% (v/v) acetic acid and subjected to microwave power of 600 W (Thongpoem et al. 2021) with different irradiation times (X_2) (Table 1). All the flasks were incubated at 50 °C without shaking for 6 h and total soluble solids (TSS) and total phenolic content (TPC) were measured. Statistical analysis of the data was performed using TIBCO® Statistica™, as described by Lomthong et al. (2022). The identified optimal mushroom concentration (X_1) and microwave irradiation time (X_2) were used to validate the model in 250 mL Erlenmeyer flasks.

Upscale mushroom extraction

To upscale microwave-assisted enzymatic hydrolysis extraction, the optimal concentration of mushroom powder was dissolved in a mixture of enzyme solution in a 1.0 L beaker and the pH was adjusted to 5.5 by 5.0% (v/v) acetic acid before irradiation in a microwave oven at the optimal irradiation time. The reaction was then transferred to a glass jar chamber (18 × 18 × 28 cm) with a working capacity of 3.0 L of substrate suspension. The reaction was incubated at 50 °C without shaking for 6 h and samples were taken during interval times to determine TSS and TPC. The type of sugar syrup at different intervals was evaluated by thin-layer chromatography (TLC) following the protocol of Sassaki et al. (2008) with some modifications.

At the end of the reaction, the mushroom syrup was used to determine the amino acid profiles using high-performance liquid chromatography (HPLC) (Agilent, 1100), Model RF-10AXL fluorescence detector was used for the analysis. Standards of amino acids (Sigma–Aldrich, USA) were used in the study (Çevikkalp et al. 2016). The dietary fiber was determined using an in-house method based on AOAC by the Central Laboratory (Thailand) Co., Ltd.

A scanning electron microscope (SEM) (JEOL, JSM-5410 LV, Japan) was used to characterize the physical structure of the native and digested mushroom powder after washing twice with distilled water and drying at 50 °C for 24 h.

Table 1 Experimental levels of the two independent variables used in the central composite design (CCD)

Independent variable	Level				
	-1.414	-1	0	1	1.414
X_1 Mushroom concentration (g/L)	39.65	50	75	100	110.35
X_2 Microwave irradiation time (s)	11.72	20	40	60	68.28

Alcoholic fermentation

Alcoholic fermentation was performed in a 5.0-L glass jar chamber (18 × 18 × 28 cm) following Sangngern et al. (2020) with three concentrations of mushroom syrup diluted with distilled water (undiluted: 1:1 and 1:2). Sucrose was added to the reaction to attain the same initial concentration at 22°Brix by Pearson's square method. The process was supplemented with diammonium phosphate (DAP) at 1.2 g/L and potassium metabisulphite (KMS) at 350 ppm, with citric acid used to reduce the pH to 4.0. Before adding yeast inoculum, the reaction tanks were kept at room temperature for 24 h. *S. cerevisiae* inoculum was subjected to the fermentation tank at a ratio of 10% (v/v) and incubated under static fermentation for 21 days at 30 °C. At the end of the fermentation, samples were taken to determine TSS, TPC, pH and alcohol content.

Vinegar fermentation

The mushroom wine was used as a substrate for vinegar fermentation with *A. aceti* TISTR 354 through surface culture fermentation following the method of Saithong et al. (2017). One hundred milliliters of a starter culture of *A. aceti* TISTR 354 was added to stainless-steel trays that contained 300 mL of each mushroom wine (1:1 and 1:2 dilutions of mushroom syrup) and 600 mL of mushroom syrup (5.0°Brix). The trays were covered by plastic sheets with punched holes and incubated at room temperature (30 ± 2 °C) for 3 days. The reaction was then added to the mushroom wine at 1.0 L to continue acetic acid fermentation for 4–5 days. Samples were taken to determine alcohol content, acidity as acetic acid, TPC, and DPPH radical-scavenging activity. An inductively coupled plasma mass spectrometric (ICP–MS) and inductively coupled plasma optical emission spectrometry (ICP OES) procedures have been developed to determine trace elements or heavy metals in this study, as reported by Castineira et al. (2001) and Bressy et al. (2013). The contaminated microorganisms were determined using an in-house method based on AOAC according to the Notification of the Ministry of Public Health No.416 by the Central Laboratory (Thailand) Co., Ltd.

A headspace gas chromatography-mass spectrometer (GC–MS) (HS-20, Shimadzu, Japan) with an HP-5MS column (30 m × 320 µm, 0.50 µm) was used to analyze the volatile compounds in the mushroom vinegar, as reported by Liu et al. (2019). Helium carrier gas (purity 99.999%) was applied with a constant flow of 1.52 mL/min and the scanning range was 35 to 500 amu. The area related to the internal standard of each volatile compound was used to quantify each substance.

Analysis

Total soluble solids (TSS)

A refractometer (RA-250WE, Kyoto, Japan) was used to determine the total soluble solids (TSS) content of the samples, as reported by Sangngern et al. (2020).

Total phenolic content (TPC)

A sample aliquot of 200 µL was added to 1.0 mL of Folin-Ciocalteu reagent, diluting 1:10 with distilled water. Next, 800 µL of sodium carbonate (Na_2CO_3) and distilled water were added to create a final volume of 5 mL. After 2 h of incubation, the absorbance of the reaction was determined at 760 nm. Results were displayed as milligrams of gallic acid equivalent per gram of substrate (mg GAE/g substrate) using gallic acid as the standard (Butsat & Siriamornpun 2010).

DPPH radical-scavenging assay

The DPPH radical-scavenging activity (%) was determined following Sripo et al. (2016) by mixing 1.0 mL of DPPH with 1.0 mL of appropriately diluted samples. After shaking, the mixtures were incubated at room temperature for 1.0 h in the dark and absorbance was measured with a UV–Vis spectrophotometer at 517 nm. To compute the DPPH (%), the control reaction with a DPPH solution without a sample and a blank sample containing distilled water was employed and calculated as shown below.

$$\mathrm{DPPH}(\%) = \left[\left(\mathrm{A}_{\mathrm{control}} - \mathrm{A}_{\mathrm{sample}}\right)/\mathrm{A}_{\mathrm{control}}\right] \times 100$$

Alcohol content

The alcohol content of the samples was evaluated by an ebulliometer (Dujardin-Salleron, Paris, France), as reported by Sangngern et al. (2020) and (Kocabey et al. 2016).

pH and acetic acid acidity

A pH meter (Model 430; Corning, NY, USA) was used to measure the pH of the medium and samples, while the titratable acidity was determined as acetic acid for vinegar using phenolphthalein as an indicator and titrating the vinegar with 1N NaOH (Helrich 1990).

Acetic acid analysis

High-performance liquid chromatography (HPLC) was used to analyze acetic acid in the mushroom vinegar following the method of Mullin and Emmons (1997).

Statistical analysis

All results were calculated as mean ± SD (standard deviation). Mean values, standard deviation, and analysis of variance (ANOVA) were computed to evaluate statistically significant values.

Results and discussion

Microwave-assisted enzymatic hydrolysis extraction

The chemical composition of wild edible mushroom, *R. delica* Fr. powder (Table 2) gave high protein (30.53 ± 0.07%), hemicellulose (25.13 ± 0.04%) and cellulose (15.39 ± 0.02%) indicating potential substrate application for production of sugar syrup containing nutrient by enzymatic hydrolysis. The mushroom had high fiber (15.52 ± 0.01%) with smaller amounts of starch (0.06 ± 0.01%), fat (2.41 ± 0.05%) and lignin (1.08 ± 0.01%). Ouzouni et al. (2009) reported that *R. delica* collected from West Macedonia and Epirus, Greece contained high protein 26.10 ± 0.30%, fat 4.44 ± 0.04% and ash 5.61 ± 0.03%. Therefore, *R. delica* could be used as an essential nutritional source with high protein, carbohydrate, and mineral contents.

Response surface methodology (RSM) with central composite design (CCD) was used to investigate the two independent variables affecting sugar syrup phenolic compound extraction, including mushroom concentration (X_1) and irradiation time (X_2). The extraction process was operated at pH 5.5 and 50 °C without shaking as described in the method. These are the optimum pH and temperature for enzyme activities as recommended by the product instructions. Extraction with high enzyme activity yields a high amount of sugar syrup and total phenolic compounds, which influence the quality of the final vinegar product. Results of TSS and TPC production from each experimental run are shown in Table 3. The highest TSS and TPC values were found at the center point of the CCD (75 g/L of mushroom concentration and 40 s of irradiation time). The matrix result of CCD was subjected to STATISTICA 10 for Windows™ to analyze the data and construct second-order polynomial equations to predict the model, as shown by the following equations for Y_1 and Y_2.

$$Y_1 = -13.6075 + 0.3649X_1 + 0.2034X_2 - 0.0019X_1X_1 - 0.0016X_2X_2 - 0.0011X_1X_2$$
$$Y_2 = -1.40902 + 0.15626X_1 + 0.05421X_2 - 0.00082X_1X_1 - 0.00047X_2X_2 - 0.00016X_1X_2$$

where Y_1 and Y_2 are the predicted responses of TSS and TPC respectively and X_1 and X_2 are the coded values of mushroom concentration and irradiation time respectively.

Results of the experimental matrix were checked by the t-test and analysis of variance (ANOVA) revealed that *p*-values of mushroom concentration (X_1) and irradiation time (X_2) were 0.0008 and 0.0142 respectively for TSS production (Table 4). The *p*-value is a statistical test that determines the probability of statistical hypothesis test results, which aids in determining the significance of the study's parameters. Lower values than 0.05 indicate that these factors significantly impact the study's

Table 2 Chemical composition of wild edible mushroom, *Russula delica* Fr

Component (%)	Analysis (%)
Starch	0.06 ± 0.01
Protein	30.53 ± 0.07
Fat	2.41 ± 0.05
Fiber	15.52 ± 0.01
Ash	6.30 ± 0.04
Hemicellulose	25.13 ± 0.04
Cellulose	15.39 ± 0.02
Lignin	1.08 ± 0.01

Note: Values are averages of three determinations

Table 3 Experimental design used in the response surface methodology with two independent variables for the production of sugar syrup and TPC content: substrate concentration (X_1) and microwave irradiation time (X_2)

Run	Level		Actual level		TSS (°Brix)		TPC (mg GAE/ g)	
	X_1	X_2	X_1	X_2	Observed	Predicted	Observed	Predicted
1	0	-1.414	75	11.72	4.43 ± 0.35	4.37	6.18 ± 0.20	6.14
2	1	-1	100	50	3.93 ± 0.14	4.77	6.62 ± 0.23	6.789
3	-1	-1	50	20	2.00 ± 0.00	2.27	4.96 ± 0.09	5.10
4	1.414	0	110.35	40	4.67 ± 0.35	4.48	6.67 ± 0.31	6.59
5	-1.414	0	39.65	40	1.93 ± 0.14	1.76	4.89 ± 0.19	4.66
6	1	1	100	60	4.43 ± 0.57	3.96	6.60 ± 0.21	6.64
7	-1	1	50	60	3.00 ± 0.00	3.15	5.17 ± 0.21	5.43
8	0	1.414	75	68.28	4.03 ± 0.35	4.05	6.61 ± 0.09	6.40
9	0	0	75	40	5.67 ± 0.28	5.47	6.59 ± 0.19	6.65
10	0	0	75	40	5.53 ± 0.14	5.47	6.74 ± 0.09	6.65
11	0	0	75	40	5.53 ± 0.14	5.47	6.69 ± 0.13	6.65

Note: Values are averages of three determinations

Table 4 Summary of the analysis of variance (ANOVA)

Factor	TSS		TPC	
	T-statistic	*p*-value	T-statistic	*p*-value
Model	-5.3837	0.0029 [sig]	-1.2105	0.2801
X_1	7.0727	0.0008 [sig]	6.5769	0.0012 [sig]
X_2	3.6849	0.0142 [sig]	2.1324	0.0861
X_1^2	-5.8168	0.0021 [sig]	-5.4966	0.0027 [sig]
X_2^2	-3.1438	0.0255 [sig]	-2.0573	0.0947
$X_1 X_2$	-1.8575	0.1223	-0.5668	0.5953
R^2	0.9365			0.9594
Adjusted R^2	0.8729			0.9188

[sig] means *p*-value less than 0.05 indicating that the model term is significant at 95%

final goal (Dahiru 2008; Lomthong et al. 2022). Results suggested that mushroom concentration and irradiation time significantly affected sugar syrup production at 95% significance level ($p < 0.05$). For TPC content, *p*-values of mushroom concentration (X_1) and irradiation time (X_2) were 0.0012 and 0.0861 respectively (Table 4), suggesting that mushroom concentration had a significant effect on TPC production at $p < 0.05$. The coefficients of determination (R^2) for TSS and TPC were 0.9365 and 0.9594 respectively and acceptable for application as models in this study (Table 4).

Figure 1 shows contour and three-dimensional plots of the interaction between mushroom concentration (X_1) and irradiation time (X_2). Maximum TSS and TPC content from the hydrolysis of mushroom powder by microwave-assisted enzymatic hydrolysis extraction ranged 80 to 90 g/L mushroom concentration and 30 to 50 s irradiation time. Beyond these values, TSS and TPC fell below the optimal levels.

Microwave-assisted enzymatic hydrolysis has long been recognized as a promising and powerful method for extracting bioactive components from plant materials (Cheng et al. 2015). The main mechanisms of microwave irradiation that improve yield extraction involve ionic conduction and dipole rotation, which result in power dissipation within the solvent and substrate, subsequently causing molecular movement and heating (Chen et al. 2008). Microwave irradiation causes structural disturbances on the substrate, resulting in a larger contact area between the solid and liquid phases, with improved solvent access to essential components (Cheng et al. 2015). Xiao et al. (2008) reported that microwave irradiation time affected flavonoid extraction from *Radix Astragali,* while Cheng et al. (2015) reported that microwave irradiation time greatly impacted the extraction yield of polysaccharides from the fruit of *Schisandra chinensis* Baill. Cheong et al. (2016) also found that microwave treatment irradiation time affected the extraction yield of polysaccharides from a novel *Cordyceps sinensis*.

From the prediction equations (Y_1 and Y_2), mushroom concentration and irradiation time were optimized at 85 g/L and 40 s respectively, giving predicted values of TSS and TPC at 5.52°Brix and 6.82 mg GAE/g substrate respectively. Model validation was performed under the same conditions, with results giving 5.60 ± 0.09°Brix and 6.81 ± 0.08 mg GAE/g substrate of TSS and TPC respectively and close to the predicted values. The validation results suggested that models obtained from this study

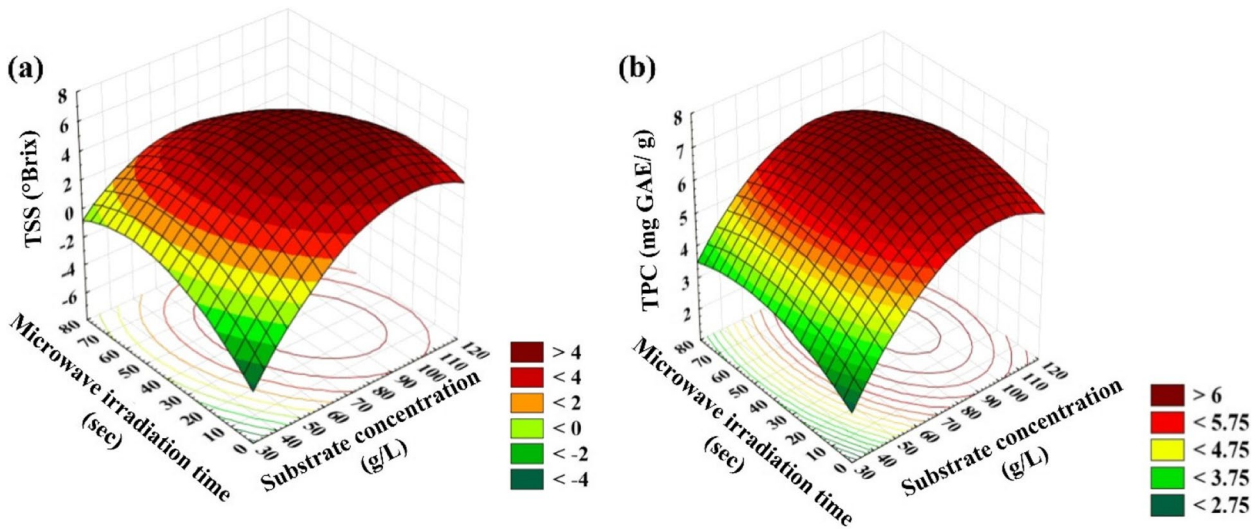


Fig. 1 Response and contour plots of the combined effects between substrate concentration (g/L) and microwave irradiation time (s) on TSS and TPC contents from the hydrolysis of mushroom powder. **a** TSS and **b** TPC

fitted and were suitable to apply for TSS and TPC production from the hydrolysis of mushroom powder by microwave-assisted enzymatic hydrolysis extraction.

Upscale mushroom extraction

The results of sugar syrup contained in phenolic compounds in a glass jar chamber with a working capacity of 3.0 L of substrate suspension are presented in Fig. 2. Total soluble solids (TSS) and total phenolic content (TPC) increased during incubation and showed a maximum at 5.60 ± 0.1°Brix and 7.01 ± 0.07 mg GAE/g substrate (Fig. 2). The main type of sugar found in the mushroom syrup was analyzed by thin-layer chromatography (TLC) as glucose, as shown in Fig. 3. The predominance of glucose in the sugar syrup, the simplest form of sugar, may support the growth of yeast and acetic acid bacteria in wine and acetic acid fermentation processes. Moreover, glucose was converted to various organic acids, alcohols, and aldehydes via the metabolic activities of yeast and acetic acid bacteria, contributing to the product's unique aroma (Lynch et al. 2019).

The amino acid profiles generated from the extraction of wild mushroom, *R. delica* are given in Table 5. Results showed that aspartic acid and glutamic acid were the main amino acids in the sample at 249.07 ± 0.01 and 230.36 ± 0.02 mg/100 g sample, respectively, with lesser amounts of serine, glycine, threonine, alanine, and proline. Glutamic acid and aspartic acid were commonly used in food as flavor enhancers. The high levels of glutamic acid and aspartic acid in mushroom syrup contribute to the good taste of the vinegar's final product. Oscar et al. (2019) published the amino acid profile of dried *R. delica*, finding 11 amino acids in the mature stage, the amount of glutamic acid was found at 0.44 mg/100 g of dried mushrooms, which is less than in this study. Dietary fiber was recorded at 2.68 g/ 100 g sample. Scanning electron micrographs of native and digested mushroom powders are shown in Fig. 4. The native mushroom powder granules were round and rough (Fig. 4a) and became swollen after irradiation (Fig. 4b). When a substrate is exposed to microwave radiation, its structure is altered, which improves solvent access to crucial internal

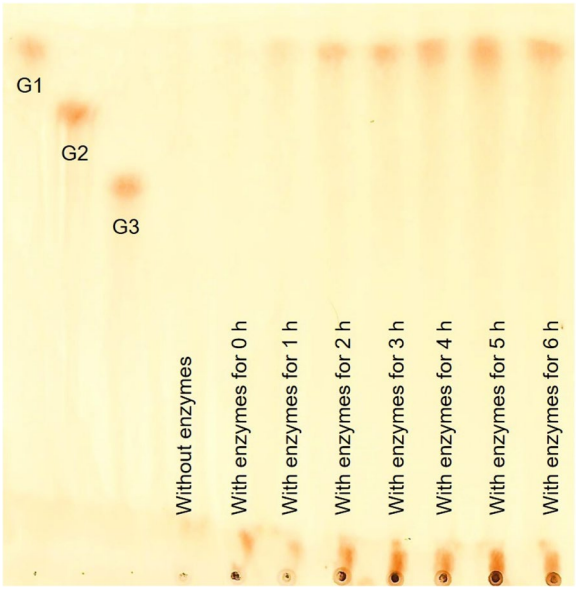


Fig. 3 TLC chromatogram of mushroom syrup after hydrolysis by the microwave-assisted enzymatic hydrolysis extraction process at 50 °C for 6 h. G1: glucose, G2: maltose and G3: maltotriose

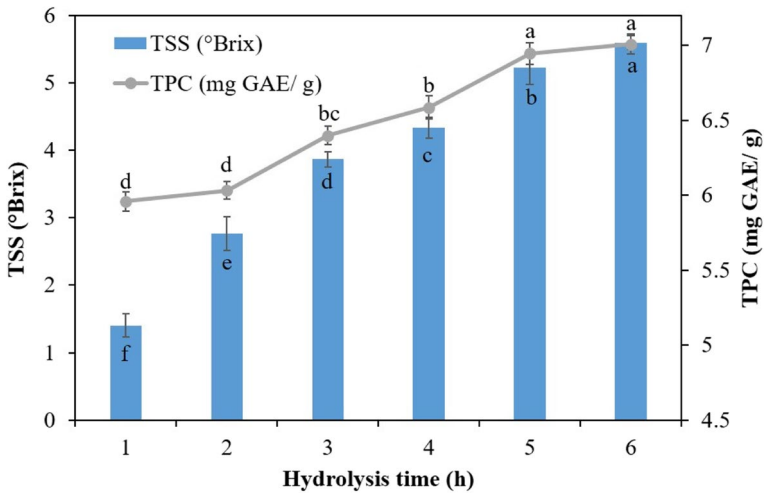


Fig. 2 Amount of TSS and TPC contents during the hydrolysis at different incubation times. Where error bars = ± SD; different lowercase letters above the bar indicate significant ($p < 0.05$) difference among means

Table 5 Amino acid profiles of wild edible mushroom, *Russula delica* Fr. after hydrolysis by the commercial enzyme at 50 °C for 6 h

Amino acid	Content (mg/100 g sample)	Amino acid	Content (mg/100 g sample)
Aspartic acid	249.07 ± 0.01	Tyrosine	113.27 ± 0.01
Glutamic acid	230.36 ± 0.02	Valine	109.0 ± 0.02
Serine	< 100	Methionine	ND
Glycine	147.52 ± 0.01	Cystine	ND
Histidine	< 100	Isoleucine	< 100
Arginine	< 100	Leucine	103.76 ± 0.01
Threonine	< 100	Phenylalanine	< 100
Alanine	< 100	Lysine	< 100
Proline	< 100	Tryptophan	186.24 ± 0.01
Hydroxylysine	ND	Hydroxyproline	ND

Note: *ND* Not Detected

components (Cheng et al. 2015). The mushroom powder then swelled as a result of the radiation. (Fig. 4b). The reaction was then incubated at 50 °C for the hydrolysis by mixed enzyme after the microwave oven irradiation was completed. The granule structure was destroyed after enzyme treatment for 3 and 6 h (Fig. 4c-d), confirming the potential of the microwave-assisted enzymatic hydrolysis process.

Alcoholic fermentation

The mushroom syrup was diluted with distilled water to determine the effect of bioactive compounds on the quality of wine production and the possibility of cost reduction. Results showed that the highest alcohol content at 10.95 ± 0.21% was obtained from the fermentation of diluted (1:1) mushroom syrup, as shown in Fig. 5a. Total phenolic content (TPC) and DPPH radical-scavenging activity were at 1.28 ± 0.23 mg GAE/mL and 69.30 ± 2.48% respectively. Undiluted syrup showed lower alcohol content (5.20 ± 0.28%) due to the high amount of antioxidative compounds (80.26 ± 1.48% DPPH radical-scavenging activity), which impacted the growth and fermentation of the yeast strain, while the undiluted mushroom syrup was viscous, affecting mass transfer in the reaction. Türkoğlu et al. (2007) reported that *R. delica* extracts showed high DPPH free radical scavenging activity (207.09 µg/mL) and high total phenolic content (47.01 ± 0.29 µg/mg pyrocatechol equivalents), total flavonoid content (8.71 ± 0.56 µg/mg quercetin equivalents) and antimicrobial activity against various microorganisms including pathogenic yeast specie, while Yaltirak et al. (2009) reported antimicrobial activity against some of the tested foodborne and spoilage bacteria and found catechin, rutin, caffeic acid and gallic acid as the main phenolics of *R. delica* ethanolic extract. The high concentration of phenolic compounds in undiluted mushroom syrup may

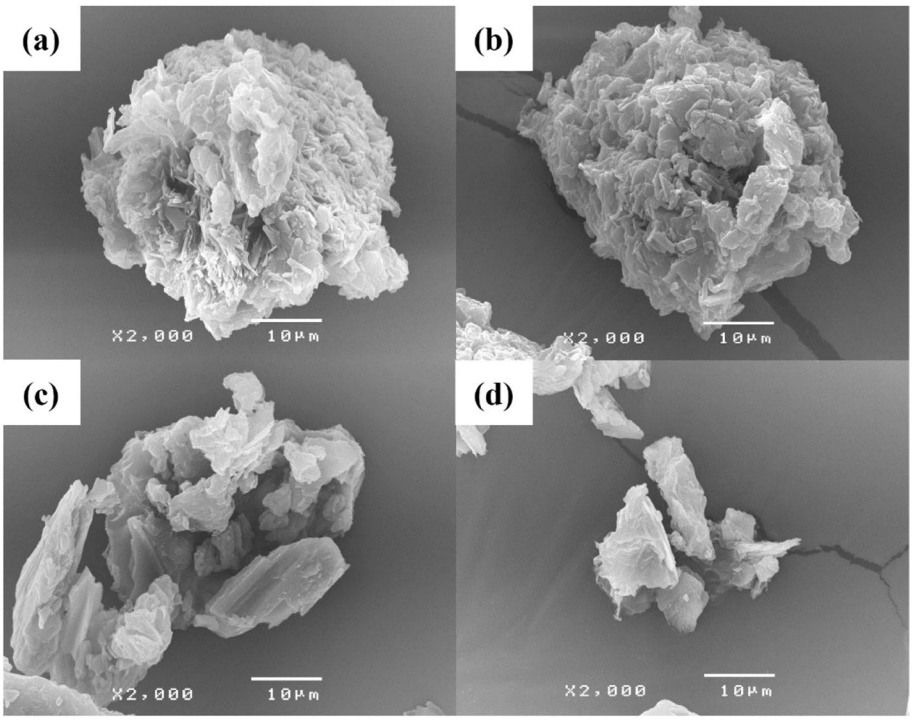


Fig. 4 Scanning electron micrographs of native and digested mushroom powder. **a** Native, **b** After microwave irradiation, **c** After hydrolysis for 3 h. and **d** After hydrolysis for 6 h

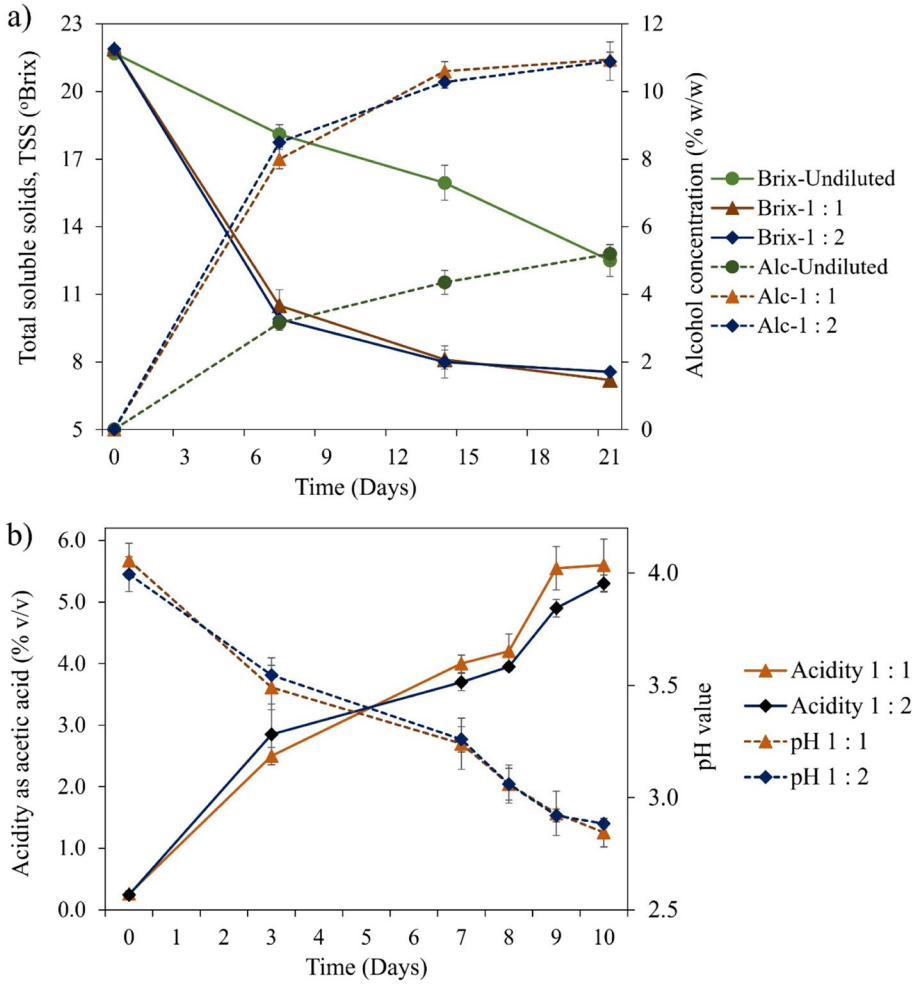


Fig. 5 Change of chemicals during alcoholic (**a**) and vinegar fermentation (**b**) from wild edible mushroom

have an impact on alcoholic yeast growth, which would lower the amount of alcohol that is produced during wine fermentation. Therefore, mushroom wines from the fermentation of diluted mushroom syrup (1:1 and 1:2) were used as substrates for vinegar fermentation.

Vinegar fermentation

The maximum acidity as acetic acid was found at 5.60 ± 0.42% w/v when using diluted mushroom wine (1:1 of mushroom syrup), as shown in Fig. 5b, while TPC was 1.87 ± 0.14 mg GAE/mL with 74.85 ± 1.24% DPPH radical-scavenging activity. The acetic acid and alcohol content were qualitatively and quantitatively analyzed by HPLC as described above at 5.28% with 0.4% alcohol content, indicating that acetic acid was the main organic acid in mushroom vinegar. The mushroom vinegar contained tryptophan, glutamic acid, aspartic acid and proline as the main amino acids at 86.24 ± 0.01, 34.69 ± 0.02, 29.55 ± 0.01 and 23.35 ± 0.01 mg/100 mL, respectively (Table 6). Liu et al. (2019) found that glutamic acid and aspartic acid contributed to the umami flavor of vinegar, while proline contributed to the sweet flavor. Vinegar derived from the wild edible mushroom, *R. delica* was a novel functional seasoning product containing amino acids, phenolic compounds and radical scavenging bioactivity for use in the food industry.

The heavy metals and contaminated microorganisms in mushroom vinegar are shown in Table 7. Arsenic (As), lead (Pb), mercury (Hg) and tin (Sn) were not found, while copper (Cu) and zinc (Zn) were present in small amounts (1.42 and 7.58 mg/L respectively) and less than the standard values of the Thai Ministry of Public Health. Ouzouni et al. (2009) reported that the wild edible mushroom, *R. delica* contained small amounts of Cu and Zn at 51.71 ± 0.30 and 56.58 ± 0.54 mg/kg respectively in the dried fruiting body, while Çayır et al. (2010) found that *R. delica* contained Cu and Zn at 37.07–164.2 and 33.45–100.17 mg/kg respectively. For contaminated microorganisms, all total plate counts, and pathogenic bacteria met the standard values of microorganisms in foods (No. 416).

Table 6 Amino acid contents of wild edible mushroom vinegar

Amino acid	Content (mg/100 mL)	Amino acid	Content (mg/100 mL)
Aspartic acid	29.55 ± 0.01	Tyrosine	11.69 ± 0.01
Glutamic acid	34.69 ± 0.02	Valine	10.61 ± 0.01
Serine	18.71 ± 0.01	Methionine	ND
Glycine	15.95 ± 0.01	Cystine	ND
Histidine	ND	Isoleucine	ND
Arginine	17.67 ± 0.02	Leucine	14.06 ± 0.01
Threonine	22.16 ± 0.01	Phenylalanine	13.19 ± 0.01
Alanine	17.28 ± 0.01	Lysine	12.23 ± 0.01
Proline	23.35 ± 0.01	Tryptophan	86.24 ± 0.01
Hydroxylysine	ND	Hydroxyproline	ND

Note: *ND* Not Detected

Table 7 Chemical and microbiological compositions of the wild edible mushroom vinegar

Analysis	Result	Standard value
Heavy metal Arsenic (As)	ND	< 2.0 mg/L
Copper (Cu)	1.42 mg/L	< 2.0 mg/L
Lead (Pb)	ND	< 1.0 mg/L
Mercury (Hg)	ND	< 0.02 mg/L
Tin (Sn)	ND	< 250 mg/L
Zinc (Zn)	7.58 mg/L	< 100 mg/L
Microorganism		
Clostridium perfringens	< 10 CFU/mL	< 100 CFU/mL
Salmonella spp.	ND	ND
Staphylococcus aureus	ND	< 100 CFU/mL
Total plate count	< 1.0 CFU/mL	< 500 CFU/mL

Table 8 Volatile compounds analysis in wild edible mushroom vinegar

Retention time	Name of compound	Percentage (%)
1.267	Acetaldehyde	0.25
1.326	Ethanol	5.18
1.390	Acetone	0.10
1.673	Acetic acid	57.65
1.740	Ethyl Acetate	15.38
1.816	Isobutyl alcohol	0.48
2.449	3-Hydroxy-2-butanone	0.09
2.742	Isopentyl alcohol	0.47
2.800	2-Methyl-1-butanol	0.21
3.295	Isobutyl acetate	0.03
4.146	3-Methylbutanoic acid	0.08
4.284	2-Methylbutanoic acid	0.04
4.825	Isopentyl acetate	0.03

The GC–MS analysis identified 13 volatile compounds in wild edible mushroom vinegar, as shown in Table 8. The sample contained acids, aldehydes, ketones, esters, and alcohols. The main compounds were acetic acid and alcohol. Liu et al. (2019) reported that alcohols, acids, esters, and aldehydes contributed to the unique aroma of vinegar products. Isobutyl acetate and isopentyl acetate provided fruity and floral aromas (Liu et al. 2019). This result confirmed that vinegar produced from the wild edible mushroom, *R. delica*, showed potential as an alternative vinegar with health benefits from the high values of nutrients and antioxidants, while also being safe from contaminated heavy metals and pathogenic microorganisms.

Conclusion

This study presented the first report on applying a wild edible mushroom, *R. delica*, as a novel substrate for wine and vinegar production with high nutritional value using microwave-assisted enzymatic hydrolysis. The obtained mushroom vinegar showed high nutritional values and was rich in amino acids and antioxidant activities. A total of fourteen amino acids were found in the mushroom vinegar, with tryptophan, glutamic acid, aspartic acid, and proline being the main amino acids that have contributed to the vinegar's umami and sweet taste. The TPC content and DPPH radical scavenging activity of the obtained mushroom vinegar were found at 1.87 ± 0.14 mg GAE/mL and 74.85 ± 1.24% respectively. The heavy metals of arsenic (As), lead (Pb), mercury (Hg), and tin (Sn) were not found in the sample while less amount of copper (Cu) and zinc (Zn) were present with meet to the Thai Ministry of Public Health standards as same as the contaminated microorganisms, indicating that the novel mushroom vinegar from this study is safe and could be used in commercial applications.

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Authors' contributions
All the authors have read and approved the final version of the manuscript. PS: conceptualisation, investigation and data acquisition. JP: investigation and data analysis. SR: investigation, methodology and supervision. PP: investigation and data acquisition. PK: data analysis and supervision. TL: conceptualisation, investigation, data acquisition and writing the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author upon a reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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