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Thai Rice Vinegars: Production and Biological Properties

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Abstract: Four types of traditional Thai rice—polished, black fragrant, glutinous and black glutinous rice—were separately used as raw material for vinegar production. During alcohol fermentation, using enriched baker's dried yeast (*S. cerevisiae*) as a starter culture gave the highest ethanol content over 7 days of fermentation. The conversion of ethanol to acetic acid for vinegar production by *Acetobacter pasteurianus* TISTR 102 was performed for 25 days. The highest amount of acetic acid was detected with glutinous rice fermentation (6.68% *w*/*v*). The biological properties of Thai rice vinegars were determined, including the total phenolic content, antioxidant activity, antibacterial activity and cytotoxicity. Black glutinous rice vinegar exhibited the maximum total phenolic content of 133.68 mg GAE/100 mL. This result was related to the antioxidative activity findings, for which black glutinous rice vinegar exhibited the strongest activity against both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals. Cytotoxicity against the human colon cancer cell line (HT-29) provided an IC_{50} value of 74.02 μg/mL and weak activity in a mouse fibroblast normal cell line (L929) with an IC_{50} value of 171.06 μg/mL. Glutinous rice vinegar was the most effective vinegar for inhibiting pathogenic bacterial growth of both Gram-negative and Gram-positive bacteria. These results suggested that the value of total phenolic content corresponded to the anticancer activity and antioxidant activity results, while antibacterial activity depended on the acidity of rice vinegar.

Keywords: rice vinegar; fermentation; total phenolic compounds; antibacterial activity; antioxidant activity

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1. Introduction

Vinegar has been widely used as a flavoring and cooking ingredient in foods for thousands of years. Various materials such as fruits, rice and whole grains are used for vinegar production via the solid-state fermentation process [1]. There are many advantages of vinegar, especially in medicinal applications, such as anti-infective, antidiabetic and cardiovascular-protective effects, digestive system assistance, appetite stimulation, regulation of blood pressure and recovery from exhaustion. Moreover, the antioxidative activity and antitumor promoting effects are found in the extracts of vinegar. Therefore, vinegar has been consumed for medicinal use and health benefits [2–4].

Rice vinegar is a traditional vinegar and has been widely used as a food seasoning for a long time in Asian countries that can be made from species of rice by solid-state fermentation. Rice vinegar is available in "white" (light yellow), red and black varieties [3,5]. Rice vinegar is classified by types of rice materials. White rice vinegar or polished rice vinegar has been known as Komesu in Japan, is colorless, has a mild acidity and has a rather smooth flavor. Some varieties of rice vinegar are sweetened or otherwise seasoned

with spices or other added flavorings [6]. In Southern China, Zhenjiang aromatic vinegar produced from sticky rice is the most famous variety. Red rice vinegar is traditionally used in China, also known as Fujian red rice vinegar. Red rice vinegar is produced from red yeast rice or red koji by *Monascus* sp. [7]. Black rice vinegar, also known as Kurosu in Japan, is produced from unpolished rice with rice germ and bran. Kurosu is dark in color and contains more nutrients such as amino acids and vitamins than Komesu. As Kurosu is rich in nutrients, it is widely used in beverages for its health benefits [2,6]. Another type of black rice vinegar derived from black glutinous rice is very popular in China, as well as in other East Asian countries. Black vinegar is characterized as a healthy drink because it has many reported health benefits [4].

Solid-state fermentation of rice vinegar consists of four continuous steps; the first step is Koji preparation. Koji is produced by inoculation of *Aspergillus oryzae* on the steamed rice to produce enzymes and other metabolites for rice vinegar fermentation [5,8]. This step is also called saccharification, which converts starch into α-amylose and sugar such as glucose and other oligosaccharides. The released sugar from saccharification is converted to alcohol (ethanol) by *Saccharomyces cerevisiae* during alcohol fermentation. This is the second step of rice vinegar fermentation. The third step is acetic acid fermentation, in which ethanol is oxidized to acetic acid by *Acetobacter* sp. After the formation of acetic acid, the maturation or ripening process is done to store the vinegar product at a low temperature for 2–3 months (depending on the type of vinegar).

Biological activity studies of vinegar refer to the total phenolic compounds and phytochemical contents of plant materials. For example, balsamic vinegar is a traditional vinegar from Italy that is made from grape or grape wine. Antioxidant compounds (phenolic acids, flavanols, polymeric tannins and melanoidins) are found in traditional balsamic vinegars [9]. Japanese rice vinegars consist of dihydroferulic acid (DFA), dihydrosinapic acid (DSA), ferulic acid and sinapic acid, which are phenolic compounds [10]. Many kinds of Chinese vinegars are made from various materials, such as Shanxi vinegar (made from sorghum), Zhenjiang vinegar (made from sticky rice), Sichuan bran vinegar (made from 108 types of medicinal herbs) and Jiangzhe rose vinegar (made from blossoming rose and rice) [7,11]. In Thailand, there are many varieties of nutrient-rich rice in all parts of the country that can be used as a material for nutrient-rich rice vinegar production. White or polished rice is popularly consumed. However, unpolished rice has become more popular due to health concerns, as its membrane contains many nutrients and trace elements. Recently, various varieties of unpolished rice have been shown to contain pigments, such as black rice, red rice, brown rice and purple rice [12,13]. Many studies have indicated that these colored rice varieties contain more nutritional advantages than white or polished rice, such as proteins, vitamins and minerals. Furthermore, the presence of dietary fibers and important phytochemicals, such as essential oils, flavonoids, phenolic compounds and anthocyanins, have also been reported in colored rice [14–16]. Many studies have described the biological properties of Thai colored rice. The phenolic compounds and pigments in Thai rice are indicated as effective antioxidative compounds. Studies of Thai colored rice, such as black glutinous rice (Niew Dam) and black fragrant rice (Hom Nil), have revealed a relation between the amount of anthocyanins and the antioxidative activity. The greater the amount of anthocyanins, the more antioxidative activities are detected. Furthermore, the content of anthocyanins in Thai colored rice is higher than non-colored or polished rice [13]. Additionally, black glutinous rice has been reported as the colored rice containing the highest amounts of anthocyanins in eight studied rice samples. The anthocyanins detected in black glutinous rice are cyanidin 3-glucoside and peonidin 3-glucoside [15]. Moreover, phenolic compounds such as ferulic acid, p-coumaric and vanillic acid are found in Thai black glutinous rice. Other biological effects of colored rice such as antimutagenic activity, anticancer activity, inhibition of allergic reactions, reduction of cardiovascular disease development and decreased plasma levels of cholesterol have been reported [14,16,17].

Although rice vinegar is widely used not only for food seasoning, but also health benefits in many Asian countries such as China and Japan, production of rice vinegar and using rice vinegar for health benefit in Thailand are not popular as expected. This study aims to produce vinegar from four cultivars of traditional Thai rice: glutinous rice (Kiaw Ngu), black glutinous rice (Niew Dam), polished rice (Hom Mali) and black fragrant rice (Hom Nil), using solid-state fermentation. This method offers many advantages such as simple, inexpensive, high yield of productivity and suitable for many agricultural product utilizations including vinegar fermentation from rice. The biological properties of produced vinegars such as antibacterial activity, antioxidant activity and anticancer activity are determined to enhance the quality on health benefits of rice vinegars. Furthermore, rice vinegar production might be an alternative way for the utilization of traditional Thai rice.

2. Materials and Methods

2.1. Rice Samples

Four types of Thai rice grain—*Oryza sativa* such as polished rice (Hom Mali) and black fragrant rice (Hom Nil) and *Oryza sativa* var. glutinosa: glutinous rice (Kiaw Ngu) and black glutinous rice (Niew Dam)—were purchased from a local market in Chiang Mai for use in the fermentation of rice vinegar.

2.2. Microorganisms

Aspergillus oryzae TISTR 3018, *Saccharomyces cerevisiae* TISTR 5049 and *Acetobacter pasteurianus* TISTR 102 were purchased from Thailand Institute of Scientific and Technological Research (TISTR). Baker's dried yeast powder (*Saccharomyces cerevisiae*) was purchased from Greathill Co., Ltd., Bangkok, Thailand.

2.3. Rice Vinegar Fermentation

The fermentation of rice vinegar consisted of 4 steps: Tanekoji preparation, saccharification, alcohol fermentation and vinegar production (acetic acid fermentation).

2.3.1. Tanekoji Preparation

Tanekoji (spores of *Aspergillus oryzae*) may be regarded as an enzyme-containing starch material for starch hydrolysis. Tanekoji was prepared by adding 7.0 mL of a spore suspension of *Aspergillus oryzae* TISTR 3018 to the polypropylene bag containing 100 g of steamed rice (Hom Mali). The mixture was incubated at room temperature (29 $\pm$ 2 °C) for 3–5 days until spore formation occurred on the surface of the steamed rice. The spores grown on rice called Tanekoji were dried at 45 °C and blended before being stored in the refrigerator until use.

2.3.2. Saccharification

Saccharification of rice vinegar production is used convert starch to glucose. Tanekoji was added to cooking rice samples by 1% (w/w) of rice weight. The fermentation was performed in a glass jar with 1000 mL of working volume at room temperature (29 $\pm$ 2 °C) for 3 days of saccharification. The reducing sugar content was determined over 3 days of saccharification using the dinitrosalicylic acid (DNS) Method.

- DNS method

Three milliliters of suitably diluted fermented broth was added to a test tube, followed by 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent. The reaction mixture was boiled for 5 min and cooled to room temperature. The absorbance was measured using a spectrophotometer at 540 nm. The amount of reducing sugar was investigated using glucose as a standard solution.

2.3.3. Alcohol Fermentation

Baker's dried yeast was activated in 1.8% (w/v) glucose at room temperature (29 $\pm$ 2 °C) for 6 h and only yeast cells separated by centrifugation at 1300 rpm for 5 min before addition.

After 3 days of saccharification, 600 mL of sterile distilled water was added to 200 g of the rice sample followed by 0.5% (*w*/*w*) of baker's dried yeast (*Saccharomyces cerevisiae*). The conversion of sugar to ethanol was performed at room temperature (29 ± 2 °C) for 7 days. The fermentation broth was collected for determination of reducing sugar by DNS and ethanol content by GC with head space.

2.3.4. Vinegar Production (Acetic Acid Fermentation)

To prepare the acetic acid bacterial suspension, *Acetobacter pasteurianus* TISTR102 was cultured in glucose yeast extract agar (GYEA) slants for 4 days. Sterilized water was added to 3 agar slant tubes containing total cell counts of 2×10^8 cells and then mixed thoroughly with bacterial colonies and transferred to glucose yeast extract broth (GYEB). The bacterial cells were then incubated for 6 h at room temperature, after which the culture medium was removed by centrifugation at 1300 rpm for 5 min. The sediment of bacterial cells was eluted with fermented broth and added to a fermentation tank for acetic acid fermentation.

After 7 days of alcohol fermentation, *A. pasteurianus* TISTR102 cells cultivated for 6 h in GYEB were removed from the broth by centrifugation at 1300 rpm for 5 min. The cells were then inoculated into the fermenter for conversion of alcohol to acetic acid. The fermentation broth was collected to evaluate the reducing sugars by DNS, ethanol content by GC head space and total acid content by titration.

After acetic acid fermentation of the Thai rice vinegars with filtration and pasteurization at 62.8–65.6 °C for 30 min, they were added to sterile glass bottles. The acetic acid content in Thai rice vinegars was determined by High-performance liquid chromatography (HPLC).

2.4. Determination of Alcohol Concentration by Gas Chromatography (GC) Head Space

The ethanol concentration was determined by gas chromatography (head space), and n-propanol was used as internal standard. Gas chromatography equipped with a flame ionization detector (FID) was performed at 280 °C with an H_2 flow rate of 40 mL/min and air flow rate of 450 mL/min. The analytical column, J&W Scientific-INNOWAX (19091N-133) with dimensions of 30 m × 0.25 mm i.d. and a 0.25 μm film thickness were used at a column temperature of 80–110 °C. The GC system had an injector temperature of 200 °C. The inlet split ratio was 200/1 at 200 °C. The oven temperature program was set at 80 °C for 4 min, increasing 15 °C/min to 110 °C with a final hold time of 7 min. The head space condition was adjusted to the GC cycle time of 15 min. The vial equilibrium time was set to 3 min and vial pressure to 8.4 psi. The pressure time, loop fill time, loop equilibrium time and injection time were set at 0.1 min. The headspace oven temperature, sample loop temperature and transfer line temperature were fixed at 80 °C, 90 °C and 100 °C, respectively. Nitrogen was used as the carrier gas with a flow rate of 1 mL/min with 7.0 psi.

2.5. Determination of the Total Acid Concentration by Titration

The total acid content was determined by titration of fermented broth with 0.1 M NaOH using phenolphthalein as an indicator.

2.6. Determination of the Acetic Acid Concentration by High-Performance Liquid Chromatography (HPLC)

The acetic acid content of Thai rice vinegars was determined by HPLC (Agilent HP 1100) with a HP hypersil ODS 4.0 × 250 mm column and UV detector 210 nm. Chromatography was performed using 0.02% formic acid as a mobile phase at a flow rate of 1 mL/min with a sample injection volume of 5 μL.

2.7. Improvement of the Fermentation Process for Vinegar Production

According to the acetic acid produced from ethanol, the ethanol content from alcohol fermentation should be sufficient for vinegar production. Therefore, the efficiency of yeast

inoculum as a starter culture is aimed to investigate. However, the preparation of yeast as a starter culture should be convenient and produce high contents of ethanol in a short fermentation period. For starter culture improvement, 2 types of yeast inoculums: baker's dried yeast and suspension of *S. cerevisiae* TISTR 5049 (4×10^7 cells), were compared. To prepare the yeast suspension, *S. cerevisiae* TISTR 5049 was cultivated at room temperature (29 ± 2 °C) on a yeast extract–malt extract agar (YMA) slant for 3 days. Seven milliliters of sterilized DI water were added to each agar slant and mixed thoroughly with yeast colonies. For inoculation, 2 tubes of an agar slant with 14 mL of suspension (4×10^7 cells) were used for each fermentation tank. Baker's dried yeast of 0.5% (*w*/*w*) was activated in 1.8% (*w*/*v*) glucose (0.1 M) at room temperature (29 ± 2 °C) for 6 h for enrichment of dried yeast. Non-enriched inoculum was not activated in glucose solution before used. These conditions were used to compare ethanol production in the alcohol fermentation step.

2.8. Biological Properties of Rice Vinegar Determination

2.8.1. Total Phenolic Compound Determination

The total phenolic content was determined by the Folin–Ciocalteu method. Three milliliter of diluted rice vinegar samples were mixed with 5 mL of Folin–Ciocalteu's phenol reagent and left for 3 min at room temperature (29 ± 2 °C), followed by the addition of 2 mL of 7.5% (*w*/*v*) Na_2CO_3. The mixtures were allowed to stand for 60 min in the dark, and the absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per 100 mL of rice vinegars (mg GAE/100 mL).

2.8.2. Antibacterial Activity Determination

The antibacterial activity of both Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were determined in the rice vinegar samples. The tested microorganisms were cultivated on nutrient agar at room temperature (29 ± 2 °C) for 24 h before use. Then, they were grown in 10 mL of nutrient broth at room temperature for 8 h, and the cell concentration was adjusted to 0.5 McFarland (10^6–10^8 CFU/mL). The antibacterial activity was investigated by measuring the diameter of the inhibition zone (DIZ). Positive results were demonstrated as clear zones resulting from bacterial growth inhibition by rice vinegar samples.

2.8.3. Cytotoxic Activity Determination

The cytotoxicity of rice vinegars against the cancer cell line was determined by the MTT assay [18] using HT-29 (human colon adenocarcinoma) and L929 (mouse fibroblast normal cell line), which were provided from Department of Biomedical Sciences, Faculty of Medicine, Prince of Songkhla University, Thailand. HT-29 (human colon adenocarcinoma) were kindly donated by Associate Professor Dr. Surasak Sangkhathat (Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand). Normal fibroblast cells (L929) were provided by Assoc. Prof. Dr. Jasadee Kaewsrichan (Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand). The testing cell lines were seeded in 96-well plates at 2×10^4 cells/well for 24 h. The cells were then incubated with rice vinegar samples at various concentration for 72 h. Next, 100 µL of 0.5 mg/mL MTT was added to each well and incubated at 37 °C for 30 min. After incubation, the dark blue crystals of formazan (MTT metabolites) were dissolved in 100 µL of dimethyl sulfoxide (DMSO) and incubated at 37 °C for 30 min. The absorbance representing the level of reduced MTT was measured at wavelengths of 570 and 650 nm using the microplate reader. The cytotoxic activities of rice vinegars against the cancer cell line were considered as the IC_{50} value.

2.8.4. Antioxidant Activity Determination

ABTS and DPPH methods were used to determine the antioxidant activity of Thai rice vinegars. The reaction times at 50% ABTS and DPPH remaining in each type of rice

vinegar were determined every 10 s for 300 s (5 min). The commercial fermented rice vinegar (5%) and commercial distilled vinegar (4.2%) were purchased from a local market in Chiang Mai province, Thailand, to compare the antioxidant activity with four types of rice vinegar sample.

- DPPH method

The assay mixture contained 1 mL of 0.2 mM DPPH radical solution in 95% ethanol and 0.5 mL of the diluted rice vinegar sample. The absorbance was measured at 515 nm every 10 s for 5 min and reported as the remaining percentage of DPPH radical scavenging according to the following formula.

$$\%\text{remaining} = \left(\frac{A_{515} \text{ at that time}}{A_{515} \text{ at 0 s}}\right) \times 100 \quad (1)$$

- ABTS method

The assay mixture contained 1.96 mL of ABTS and 0.04 mL of the rice vinegar sample. The absorbance was measured at 734 nm every 10 s for 5 min and reported as the remaining percentage of ABTS radical scavenging according to the following formula.

$$\%\text{remaining} = \left(\frac{A_{734} \text{ at that time}}{A_{734} \text{ at 0 s}}\right) \times 100 \quad (2)$$

2.9. Statistical Analysis

The data were expressed as the mean ± SD of three replicate independent experiments and analyzed by one-way and two-way analysis of variance (ANOVA), Duncan's multiple range-test and an independent T-test to determine significant differences. p values < 0.05 were regarded to be statistically significant. Statistical Package for the Social Sciences (SPSS) version 16.0 by IBM Company was used for statistical analysis in this study.

3. Results and Discussion

3.1. Comparison of the Starter Culture in Alcohol Fermentation

The yeast suspension of *Saccharomyces cerevisiae* TISTR 5049, non-enrichment and enrichment of baker's dried yeast activated in 1.8% (w/v) glucose were used as a starter to compare ethanol productivity in alcohol fermentation. The results showed that the maximum ethanol content was produced by enrichment of baker's dried yeast fermented with average content of four types of rice sample over 7 days of fermentation (Figure 1). The yeast suspension and non-enrichment of baker's dried yeast used as starter cultures could produce a lower amount of ethanol than enriched baker's dried yeast. The ethanol productivity using yeast suspension and non-enrichment of baker's dried yeast as a starter culture in four types of rice fermentation showed no statistically significant difference (Table 1).

Table 1. Ethanol production of each rice substrate using various starter cultures.

Yeast Culture	Ethanol Content (%v/v)			
	Polished Rice	Black Fragrant Rice	Glutinous Rice	Black Glutinous Rice
Yeast suspension	3.17 ± 0.07^{cB}	3.46 ± 0.13^{cB}	4.12 ± 0.08^{aB}	4.07 ± 0.12^{bB}
Baker's dried yeast (non-enrichment)	3.58 ± 0.04^{cB}	3.22 ± 0.07^{cB}	4.45 ± 0.12^{aB}	3.84 ± 0.10^{bB}
Baker's dried yeast (enrichment)	4.95 ± 0.12^{cA}	5.16 ± 0.12^{cA}	5.98 ± 0.09^{aA}	5.54 ± 0.12^{bA}

Values are expressed as the mean ± S.D from triplicate assessments. $^{A–B}$ Columns followed by different letters are significantly different in the starter culture of yeast ($p < 0.05$). $^{a–c}$ Rows followed by different letters have significantly different ethanol contents ($p < 0.05$).

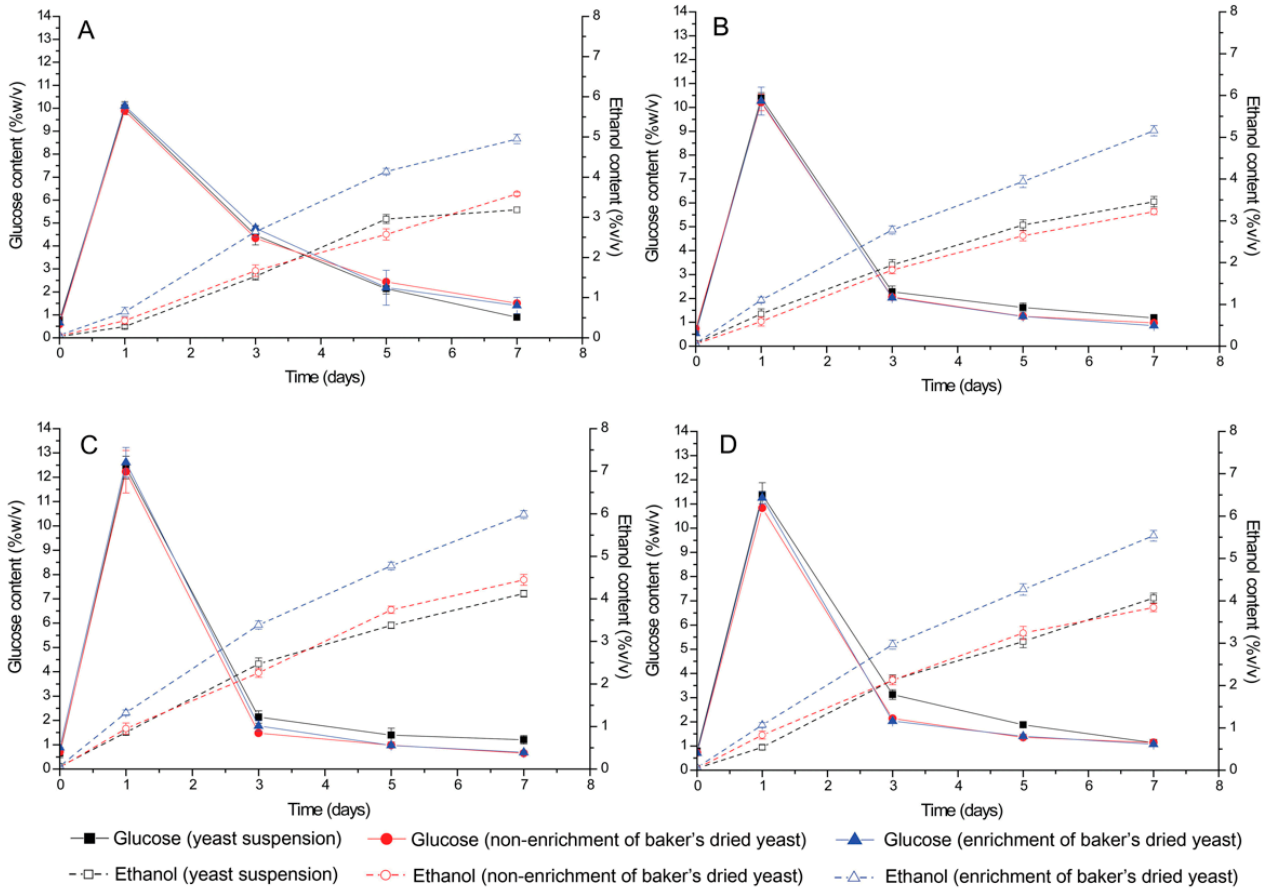

Figure 1. Alcohol fermentation from polished rice (**A**), black fragrant rice (**B**), glutinous rice (**C**) and black glutinous rice (**D**) with various starter cultures.

The glucose content was decreased after the first day of alcohol fermentation due to conversion to ethanol (Figure 1). The released glucose from saccharification was converted to ethanol by yeast, and it was consumed for cell division and growth of yeast culture in the fermentation broth. These results showed that the glucose content was diminished immediately after the first day of fermentation.

In a previous study, baker's yeast and loog-pang (a Thai traditional fermentation starter culture) were used in the hydrolysis of oil palm empty fruit bunch aqueous phase enriched with glucose culture for ethanol fermentation. The results showed that the ethanol content produced by baker's yeast was higher than that by loog-pang [19]. Furthermore, alcohol fermentation of commercial dried yeast was studied. Commercial New Aule alcohol yeast and New Aule baker's instant dried yeast were used for bioethanol production in sugarcane molasses. The New Aule baker's instant dried yeast produced more ethanol than its alcohol yeast with an ethanol content of 102.854 g/L and 74.8 g/L, respectively. Commercial yeast strains have many advantages compared with natural yeast strains, such as a longer shelf life (more than one year), high cell viability (up to 4.6×10^{10} yeast cells/g), a high efficiency for culture in the short term, low or no contamination from other microbes, lower cost and predominant properties such as high thermal, sugar, alcohol and acid tolerance [20].

Thus, baker's dried yeast was used as a starter culture in the alcohol fermentation step due to the simple and more convenient. The ethanol productivity from enrichment of baker's dried yeast also showed a high efficiency in the further step of acetic acid fermentation.

3.2. Solid State Fermentation of Rice Vinegar

Four types of Thai rice—polished rice (Hom Mali), black fragrant rice (Hom Nil), glutinous rice (Kiaw Ngu) and black glutinous rice (Niew Dam)—were used as raw materials for vinegar fermentation.

The fermentation broth was sampled to determine the glucose, ethanol and total acid content during fermentation period; the results are shown in Figure 2.

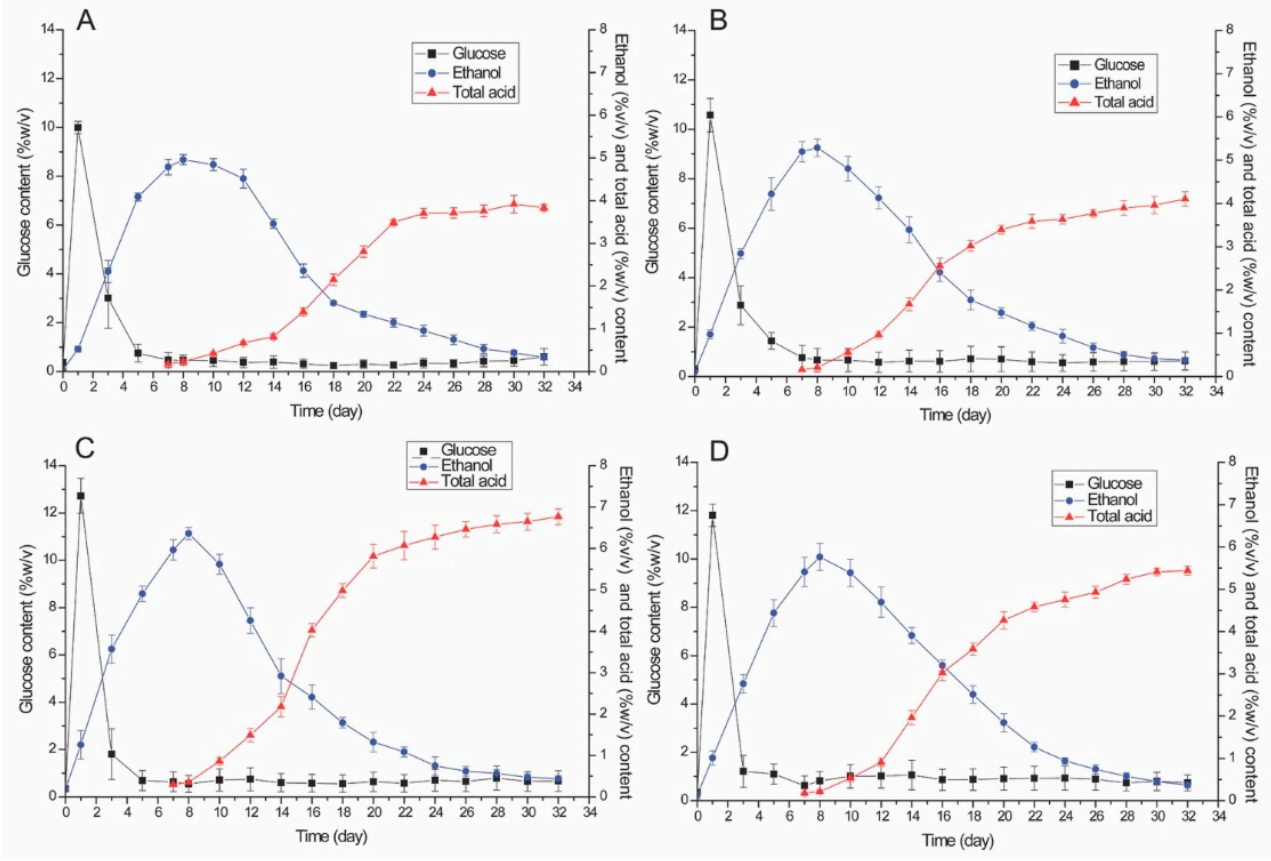

Figure 2. Total acid content in fermented broth of polished rice (**A**), black fragrant rice (**B**), glutinous rice (**C**) and black glutinous rice (**D**) during vinegar fermentation.

The glucose content in fermentation broth was determined after 3 days of saccharification. Subsequently during alcohol fermentation, glucose content was detected at low levels due to the recent addition of water to the fermenter; thus, a small amount of glucose from saccharification was dissolved in the broth. On the next day, the maximum glucose content was detected on the first day of alcohol fermentation. The fermentation broth of glutinous rice exhibited the highest content of glucose at 12.66% (*w*/*v*), followed by black glutinous rice, black fragrant rice and polished rice with maximum glucose contents of 11.42%, 10.07% and 9.80% (*w*/*v*), respectively. The glucose content then decreased rapidly during the next period, and the ethanol content increased. These results suggested that glucose from saccharification was consumed by yeast cells and converted to ethanol.

The maximum ethanol contents in the four types of rice samples were detected on days 7 and 8 of fermentation and were found to decrease due to the conversion of ethanol to acetic acid. The fermentation broth of glutinous rice exhibited the highest content of ethanol at 6.21% (*v*/*v*), followed by black glutinous rice, black fragrant rice and polished rice with maximum glucose contents of 5.64%, 5.27% and 4.95% (*v*/*v*), respectively. Ethanol productivity during fermentation varied by glucose content from hydrolyzed rice starch [5].

During ethanol fermentation, a critical high concentration of ethanol can cause toxicity to yeast cells [21]. When the cell membrane of *S. cerevisiae* attaches to the accumulated

ethanol, the composition of the cell membrane or organelle structure of *S. cerevisiae* is changed for adaptation, leading to a higher tolerance to ethanol stress. Dong et al., (2015) found that *S. cerevisiae* showed a greater variation of the cell membrane in stationary phase and log phase than lag phase [22]. These results indicated that the stationary phase and log phase of *S. cerevisiae* exhibited higher ethanol tolerance. *S. cerevisiae* has been widely used in brewing and ethanol production industries, demonstrating a high tolerance to ethanol levels ranging approximately from 14% to 20% (*v*/*v*) of the brewing products [23]. However, the ethanol content detected in this study was lower, so the yeast should have remained alive and active in the fermentation system.

Ethanol was oxidized to acetic acid by *A. pasteurianus* TISTR102 after adding the cell starter culture to the fermentation broth. In this step, the total acid content was determined and increased throughout fermentation, while the detected ethanol content was decreased (Figure 2). The increase in total acid content in the last period of fermentation was slow or remained constant. Glutinous rice contained the highest total acid content at the end of acetic acid fermentation, followed by black glutinous rice, black fragrant rice and polished rice, with values of 6.77%, 5.44%, 4.10% and 3.83% (*w*/*v*), respectively. Sapinosa et al., (2015) reported the conversion of 6.28% (*w*/*v*) alcohol from rice wine to an acetic acid content of 6.6–7.8% (*w*/*v*), which indicated a high rate of ethanol conversion to acetic acid [24]. Ethanol, glycerol and Na-DL lactate are the best carbon sources for *Acetobactor* sp., especially ethanol, which is used for acetic acid production [25]. Consequently, the ethanol from the alcohol fermentation step is necessary for acetic acid fermentation.

Saccharified glucose of 200 g cooking rice showed a maximum content on the first day of alcohol fermentation. The saccharified glucose content of glutinous rice and black glutinous rice showed no statistically significant difference and was higher than the content of polished and black fragrant rice. Glucose was then converted to ethanol during this step, and the maximum ethanol production was detected after day 7 of alcohol fermentation.

Previous studies have reported an effect of conditions on acetic acid bacteria growth. The glucose tolerance of acetic acid bacteria isolated from traditional balsamic vinegar has been studied, demonstrating that the general acetic acid bacterial growth was inhibited by 25% glucose. *A. pasteurianus* was able to grow in 10% ethanol with 5% yeast extract medium [25]. The effect of ethanol on acetic acid bacterial growth in wine was evaluated and showed that the cells remained viable in the presence of 10% and 14% (*v*/*v*) ethanol. Acetic acid from ethanol oxidation is indicated as a limiting factor for acetic acid bacterial growth. The cells are affected by the high acetic acid concentration (approximately 12%) due to the decreased enzymatic activity of alcohol dehydrogenase [26].

The observed pH value change in the fermentation broth during fermentation were determined. After the addition of water in the first alcohol fermentation step, the pH was approximately 7 and then decreased to 3.06–3.51 at the end of fermentation. The optimized pH for growth of acetic acid bacteria is 5.0–6.5, although these bacteria can grow at lower pH values (3.0–4.0) such as in wine with high ethanol concentrations (10–15%). *A. pasteurianus* has been reported to show increased pH sensitivity to a high concentration of ethanol (12.5%). In general, the optimum growth temperature of acetic acid bacteria ranges from 25–30 °C, but several studies have demonstrated that acetic acid bacteria from industrial vinegar production are thermotolerant and can oxidize ethanol at high temperatures (38–40 °C) [26]. A study of a mixed culture of *S. cerevisiae* and *A. pasteurianus* reported an optimum growth temperature of *S. cerevisiae* of 28–30 °C and *A. pasteurianus* of 34–36 °C with a culture pH varying from 3.8 to 4.3 for acetic acid production [27].

During the acetic acid fermentation step, the acetic acid content decreased due to overoxidation. The accumulated acetic acid was oxidized to CO_2 and H_2O via the tricarboxylic cycle when ethanol was absent in the fermentation broth [28].

A summary of reducing sugar, ethanol, total acid and acetic acid contents in Thai rice vinegars is shown in Table 2. The reducing sugar and ethanol contents were retained in Thai rice vinegars even though fermentation was completed. There was no statistically significant difference. However glutinous rice vinegar exhibited the highest content of total

acid and acetic acid with statistically significant difference from other rice vinegars, follow by black glutinous rice vinegar. Otherwise, there was no statistically significant variation in total acid level between black fragrant rice and polished rice vinegar, but acetic acid content of black fragrant rice vinegar more than polished rice vinegar was statistically significant.

Table 2. Reducing sugar, ethanol, total acid and acetic acid contents in Thai rice vinegars in 32th day of fermentation at room temperature.

Rice Vinegars	Reducing Sugar Content (%*w*/*v*)	Ethanol Content (%*v*/*v*)	Total Acid Content (%*w*/*v*)	Acetic Acid Content * (%*w*/*v*)
Polished rice	0.60 ± 0.34	0.33 ± 0.06	3.84 ± 0.07 ^{c}	3.78 ± 0.18 ^{d}
Black fragrant rice	0.63 ± 0.36	0.38 ± 0.06	4.10 ± 0.17 ^{c}	4.06 ± 0.27 ^{c}
Glutinous rice	0.68 ± 0.44	0.44 ± 0.07	6.77 ± 0.19 ^{a}	6.68 ± 0.10 ^{a}
Black glutinous rice	0.66 ± 0.44	0.37 ± 0.07	5.44 ± 0.10 ^{b}	5.33 ± 0.31 ^{b}

Values are expressed as the mean ± S.D from triplicate assessments. $^{a–d}$ Columns followed by different letters are significantly different in each Thai rice vinegar product ($p < 0.05$). * Acetic acid content of polished rice, black fragrant rice, glutinous rice and black glutinous rice vinegar was 91.69%, 98.84%, 98.79% and 97.90% of the total acid, respectively.

Glutinous rice vinegar exhibited the highest content of acetic acid at 6.68% (*w*/*v*), followed by black glutinous rice, black fragrant rice and polished rice with values of 5.33%, 4.06% and 3.78% (*w*/*v*), respectively. The presence of acetic acid was related to the total acid content of Thai rice vinegar because it is the major organic acid in vinegar. However, several organic acids were also detected, as described in some studies of rice wine vinegar [24]. The acidity of wine vinegar must be at least 6% (*w*/*v*), and the maximum residual ethanol allowed is 1.5% (*v*/*v*), as determined by the European Union. In fact, ethanol concentrations between 0.5 and 1% are regularly maintained in vinegars to prevent overoxidation from the presence of acetic acid bacteria [29]. In Thailand, the standard of fermented vinegar indicates that the acetic acid content must not be lower than 4 g per 100 mL, and the content of ethanol is not specified. The reducing sugar and ethanol contents were still remained in Thai rice vinegar products with lower contents than the standard. The remaining reducing sugar and ethanol contents in four types of Thai rice vinegar showed no statistically significant differences.

After acetic acid fermentation was completed, the Thai rice vinegars were filtrated to remove the rice substrate and microorganism residues. Then, they were pasteurized at 62.8–65.6 °C for 30 min to remove the microorganisms.

3.3. Biological Properties of Rice Vinegars

3.3.1. Total Phenolic Content of Rice Vinegars

Black glutinous rice vinegar exhibited the highest content of total phenolics (133.68 ± 4.52 mg GAE/100 mL of rice vinegar), followed by black fragrant, polished and glutinous rice vinegar, providing a total phenolic content of 102.07 ± 3.44, 82.15 ± 2.67 and 76.81 ± 2.58 mg GAE/100 mL of the rice vinegar, respectively. The large amounts of total phenolic contents of pigmented rice vinegars were due to pigments in the rice grain such as anthocyanins [13]. Furthermore, among eight rice samples in the reviewed study, black glutinous rice was the pigmented rice containing the highest anthocyanin amounts. The anthocyanins detected in the black glutinous rice were cyanidin 3-glucoside and peonidin 3-glucoside [15]. Moreover, phenolic compounds such as ferulic acid, p-coumaric, sinapic acid and vanillic acid were found in rice [30]. These phenolic compounds in rice grains have shown biological activities such as antimutagenic, anticancer, allergic reaction inhibitory, cardiovascular disease preventative and plasma cholesterol level-reducing effects [14,16,17].

The variation of total phenolic contents during fermentation processes is shown in Figure 3. The total phenolic contents of rice vinegars increased continuously over the 32 days of fermentation. These phenolic compounds could be extracted from rice grain

by ethanol with an acidified organic solvent [16], such as anthocyanin extract from black rice [12]. Thus, during ethanol and acetic acid production, phenolic compounds consisting of rice substrates were extracted and released in the fermentation broth.

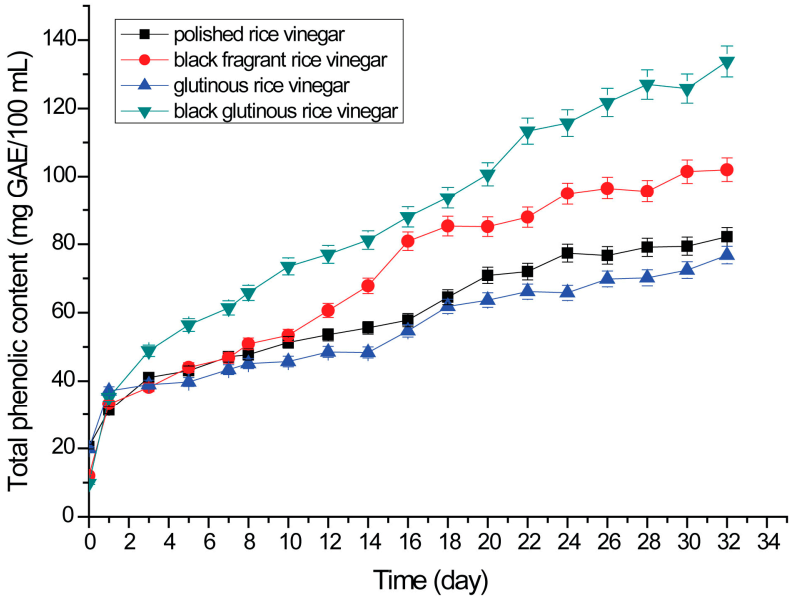

Figure 3. Total phenolic contents in rice vinegar during fermentation.

3.3.2. Determination of Antibacterial Activity

Thai rice vinegars were shown to possess antibacterial activity by the agar disc diffusion method. Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were selected as the tested microorganisms. Sterilized deionized water was used as a negative control, and antibiotics consisting of 0.24 mg/mL of tetracycline-HCl served as a positive control because of the wide antibiotic resistance of both of Gram-negative and Gram-positive bacteria [31]. After 24 h of incubation, the diameter of the inhibition zone (clear zone) was determined. Thai rice vinegar samples, commercial vinegars and acetic acid inhibited bacterial growth similarly to tetracycline-HCl. The antibacterial activities of the vinegar samples are shown as the diameter of the inhibition zone (DIZ) in Table 3.

Table 3. Diameter of the inhibition zone of Thai rice vinegars and cytotoxicity of rice vinegars toward the cancer cell lines.

Samples (Acetic Acid Content)	Diameter of Inhibition Zone (mm)			IC_{50} Value (μg/mL)	
	E. coli	*S. aureus*	*S. epidermidis*	L929	HT-29
Polished rice vinegar (3.78%)	13.7 ± 0.6^{Dd}	13.7 ± 0.6^{Dd}	10.0 ± 1.0^{Ee}	133.46	110.92
Black fragrant rice vinegar (4.06%)	14.3 ± 0.6^{Dd}	14.0 ± 0.0^{Dd}	11.0 ± 1.0^{Dde}	135.25	81.66
Glutinous rice vinegar (6.68%)	17.3 ± 0.6^{Bb}	17.7 ± 0.6^{Bb}	13.0 ± 0.0^{BCbc}	121.84	90.17
Black glutinous rice vinegar (5.33%)	17.3 ± 0.6^{Bb}	16.0 ± 0.0^{Cc}	12.0 ± 0.0^{CDcd}	171.06	74.02
0.24 mg/mL Tetracycline-HCl	21.0 ± 0.0^{Aa}	21.0 ± 0.0^{Aa}	16.7 ± 0.6^{Aa}	-	-
Commercial fermented rice vinegar (4.20%)	16.0 ± 0.0^{Cc}	16.0 ± 1.0^{Cc}	12.7 ± 0.6^{BCbc}	-	-
Commercial distilled vinegar (5.00%)	17.3 ± 0.6^{Bb}	17.3 ± 0.6^{Bb}	13.7 ± 0.6^{Bb}	-	-
Acetic acid (4.00%)	14.7 ± 0.6^{Dd}	14.3 ± 0.6^{Dd}	12.0 ± 1.0^{Dcd}	-	-
Sterilized deionized water	-	-	-	-	-

Values are expressed as the mean ± S.D from triplicate assessments. $^{A–E}$ Columns followed by different letters are significantly different in terms of the diameter of inhibition zone of Thai rice vinegars compared by acetic acid content ($p < 0.05$). $^{a–e}$ Columns followed by different letters are significantly different in terms of the diameter of inhibition zone of Thai rice vinegars compared by type of sample ($p < 0.05$).

The antimicrobial activity of the samples was classified according to the diameter of the inhibition zone as weak inhibition (7 mm < DIZ < 10 mm), medium inhibition (11 mm < DIZ < 14 mm) and strong inhibition (DIZ > 15 mm) under the statistical analysis described below Table 3 [31]. Glutinous rice vinegar was the most effective Thai rice vinegar for the inhibition of bacterial growth demonstrating strong inhibition against *E. coli* (DIZ = 17.3 ± 0.6 mm) and *S. aureus* (DIZ = 17.7 ± 0.6 mm), while showing medium inhibitory effect on *S. epidermidis* (DIZ = 13 mm). This result was similar as detected in commercial distilled vinegar. Moreover, glutinous rice vinegar showed the higher inhibitory effect on *E. coli* and *S. aureus* than commercial fermented rice vinegar significantly. The antibacterial activity of black glutinous rice vinegar on *E. coli* was similar to glutinous rice vinegar, accepted for the inhibition against *S. aureus* (DIZ = 16 mm) and *S. epidermidis* (DIZ = 12 mm) showing the lower activity significantly. Black fragrant rice vinegar demonstrated a medium inhibition against all tested bacteria (DIZ = 11–14.3 ± 0.6 mm). For polished rice vinegar, medium inhibition was detected on *E. coli* and *S. aureus* with DIZ values of 13.7 ± 0.6 mm, while weak inhibition was observed on *S. epidermidis* (DIZ = 10 ± 1 mm). However, Tetracycline-HCl (positive control) showed the strongest inhibition against all strains of bacteria tested significantly. There were no statistically significant differences of antimicrobial activity between glutinous rice vinegar and black glutinous rice vinegar. In contrast, acetic acid (4%) showed medium inhibition against all strains of bacterial tested.

Vinegars mainly consist of acetic acid have been known to possess strong antimicrobial activity against bacteria [28]. A previous study has shown that antibacterial activities of vinegars could be partly related to both their acetic acid contents and pH values, in addition to their phenolic contents. The inhibition of microbial growth or survival of bacterial cells depend on the acidity level of vinegar. Acetic acid in rice vinegar shows antimicrobial activity by traversing the cell membrane in the undissociated form and dissociating in accordance with the intracellular pH to release a proton (H^+) in the cytoplasm. The resulting enhancement of acidity in the cytoplasm leads to cell damage. Moreover, proteins and enzymes are denatured, inhibiting DNA and RNA synthesis. The energy is diminished because ATP is used to eliminate the excess protons, resulting in the growth inhibition and death of the cells [31,32].

The results of the present study indicated that all Thai rice vinegars contained antibacterial activity against both Gram-negative and Gram-positive bacteria. The antibacterial activity was related to the total acid and acetic acid contents of vinegars, which were more effective than the total phenolic content.

3.3.3. Cytotoxic Activity of Rice Vinegars

The cytotoxic activities of rice vinegars against the human colon cancer cell line (HT-29) and mouse fibroblast normal cell line (L929) were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT was reduced in mitochondria via succinate dehydrogenase to formazan products during incubation with the cell line [33]. The level of the reduced MTT form (formazan) was determined as the amount of cell line survival. The cytotoxicity was determined by the National Cancer Institute (NCI) as the 50% inhibitory concentration (IC_{50}); an $IC_{50} \leq 30$ μg/mL represent an active level. However, Geran et al., (1972) determined the level of cytotoxicity as $IC_{50} \leq 20$ μg/mL (highly active), IC_{50} 21–200 μg/mL (moderately active), IC_{50} 201–500 μg/mL (weakly active) and $IC_{50} > 501$ μg/mL (inactive) [34].

The cytotoxicities of the Thai rice vinegars are shown in Table 3. Black glutinous rice vinegar exhibited the highest cytotoxicity toward HT-29 cells with an IC_{50} value of 74.02 μg/mL. Weak activity of the vinegar against L929 cells was detected, showing an IC_{50} value of 171.06 μg/mL. Black fragrant, glutinous and polished rice vinegar also showed cytotoxicity against HT-29 cells, with IC_{50} values of 81.66, 90.17 and 110.92 μg/mL, respectively. These results indicated that Thai rice vinegars contained cytotoxic activity against colon cancer cell lines and had a diminished effect on normal cell lines. The mouse

fibroblast normal cell line (L929) cell line was used as a normal cell control, as recommended by the international standard for the testing of medical device, which demonstrates a more sensitive response than primary cell [18].

Several studies have reported the inhibitory effects of Kurosu (unpolished rice vinegar) extract on the growth of many cancer cell lines, such as colon and breast adenocarcinoma, lung, bladder and prostate carcinoma [35]. Previously, Shimoji et al., (2003) reported that ethylacetate extract of Kurosu inhibited colonic aberrant crypt foci. Kurosu also showed an inhibitory effect toward colon adenocarcinoma growth induced by azoxymethane in male rats [36]. In addition, Izumi, the Japanese black vinegar made from unpolished rice, inhibited the proliferation of a human squamous cell carcinoma cell line [37]. These anticancer activities suggested that the phenolic compounds in rice bran and germ, such as ferulic acid and its derivatives contained in unpolished rice, are the major substances inhibiting cancer cell line growth [38].

Hui et al. (2010) reported that an anthocyanin-rich extract from black rice exhibited cytotoxic effects on three human breast cancer cell lines, with IC_{50} values of 374.7, 209.9 and 179.5 μg/mL. They suggested that anthocyanin affected human breast cancer cells in vitro and in vivo by inducing apoptosis and suppressing angiogenesis [39]. Recently, sticky purple rice extract was found to prevent aberrant crypt focus (ACF) formation (the initial stage of colon carcinogenesis) in dimethyhydrazine (DMH)-induced rats [40]. Moreover, a red color strain of unpolished Thai rice (*Oryza sativa* L.) extract was investigated for its anticancer activity against ACF formation in azoxymethane (AOM)-treated rats and efficacy for apoptotic induction and oxidative redox status in human colon cancer (Caco-2) cells. They found that feeding unpolished Thai rice extract might prevent ACF formation by inhibiting precancerous progression in AOM-treated rats. They also found that unpolished Thai rice extract significantly induced cancer cell apoptosis and increased cellular oxidants in human colon cancer cells, with an IC_{50} of 5.87 mg/mL with the MTT assay at 24 h [41]. Therefore, phenolic compounds in rice grains such as phytochemicals and plant pigments are associated with anticancer activity.

Not only phenolic compounds but also acetic acid shows anticancer activity. Okabe et al., (2014) concluded that acetic acid is a powerful anticancer agent. They found that 0.5% acetic acid induced almost complete cell death in human gastric cancer and human mesothelioma cell lines. Although acetic acid was used at a low concentration of 0.1%, it was able to induce human gastric cancer cell death by 41.7% at pH 6.8 in culture medium. Thus, the authors suggested that the cytotoxic effect was induced by acetic acid directly and cell death independent of the pH of the medium [42]. Therefore, acetic acid in rice vinegar at a concentration of 4% is considered to inhibit cancer cell growth. These results indicated that the antioxidant activity was related to the total phenolic contents of the vinegar sample.

3.3.4. Determination of Antioxidant Activity

- ABTS method

The ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay depends on the blue-green color of $ABTS^{\bullet+}$ generated in the aqueous phase with a maximum absorption at 734 nm. The $ABTS^{\bullet+}$ was reduced by hydrogen donation by the antioxidant; the blue-green of $ABTS^{\bullet+}$ is converted to a colorless form and the absorption is decreased [43]. The remaining ABTS percentages in the rice vinegars are shown in Figure 4A. The blue-green of $ABTS^{\bullet+}$ was decolorized by black glutinous rice vinegar to 50% in less than 20 s, exhibiting the strongest antioxidant ability against $ABTS^{\bullet+}$. Subsequently, black fragrant, polished and glutinous rice vinegar exhibited 50% ABTS scavenging activity over 30, 120 and 220 s, respectively. In contrast to commercial fermented rice vinegar, commercial distilled vinegar and 4% acetic acid are incapable of decolorizing $ABTS^{\bullet+}$.

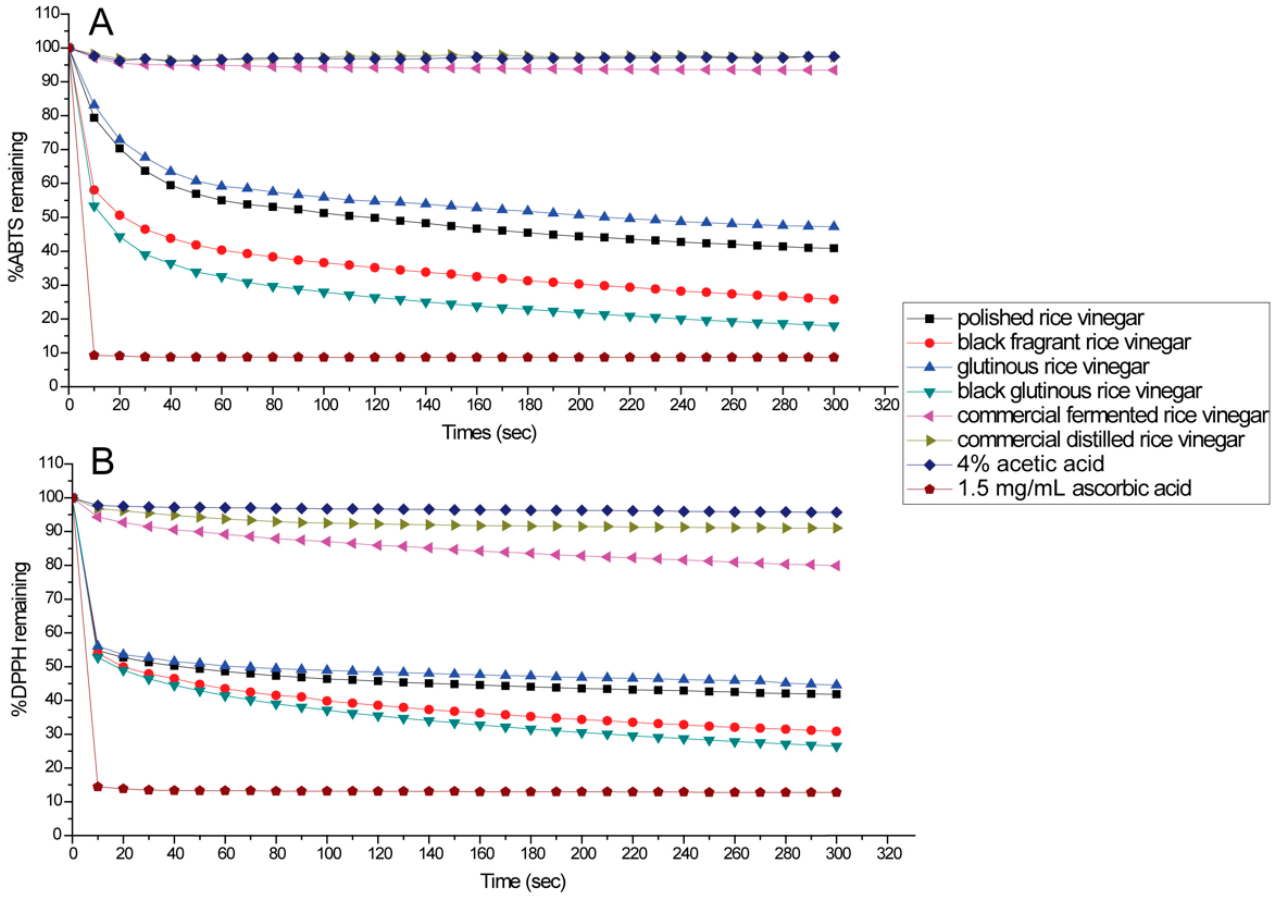

Figure 4. Antioxidant activity of rice vinegars by the ABTS method (**A**) and the DPPH method (**B**).

- DPPH method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay has been widely used to determine the antioxidant activity of foods. The DPPH• radical is a stable organic nitrogen radical with a deep purple color and the greatest absorption at a wavelength of 515 nm. The purple color of the DPPH• radical is reduced to the form DPPH-H by pairing with the hydrogen atom from antioxidant compounds, changing the color to pale yellow, which causes the absorption at 515 nm to decrease [43]. The DPPH• radical was reduced by the tested Thai rice vinegars. The results are shown in Figure 4B, wherein the DPPH• radical was reduced by black glutinous and black fragrant rice vinegar to 50% in less than 20 s. In contrast, polished and glutinous rice vinegar exhibited 50% DPPH scavenging activity for 50 and 70 s, respectively. Commercial fermented rice vinegar was unable to inhibit the DPPH• radical to 50% in 5 min but to 79.88% in 5 min. In contrast, the DPPH• radical was barely reduced by commercial distilled vinegar and 4% acetic acid.

Based on the present results, black glutinous rice vinegar showed the strongest antioxidant activity against both ABTS and DPPH, followed by black fragrant rice vinegar, polished rice vinegar and glutinous rice vinegar, respectively. The results demonstrated that all Thai rice vinegars had antioxidant activity of both $ABTS^{\bullet+}$ and DPPH• radicals. However, commercial fermented rice vinegar had slight antioxidant activity against DPPH but was ineffective with ABTS. Commercial distilled vinegar and 4% acetic acid showed no antioxidant activity. L-ascorbic acid (vitamin C) was used as a positive control for antioxidant determination. Vitamin C is a water-soluble antioxidant, which is an important reducing agent and scavenger of free radicals in biological systems. The free radical was generated with donated electron from vitamin C for stability [44]. In this study, vitamin C exhibited powerful antioxidant activity against both ABTS and DPPH (Figure 4).

The total phenolic content of commercial fermented rice vinegar was 10.55 ± 0.46 mg gallic acid/100 mL of rice vinegar, which was less than all Thai rice vinegars in this study. Thus, the total phenolic content was insufficient for inhibiting the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicle, while commercial distilled vinegar and 4% acetic acid were not detected. Thus, they showed no antioxidant activity. The antioxidant activity results were related to the total phenolic content of the vinegars. The two processes of vinegar production, include traditional and submerged (industrial), were studied, using the same type of raw material for vinegar production. The vinegar from the traditional method showed a higher total phenolic content compared with the submerged method. The industrial process resulted in nutrition loss from raw materials, so the antioxidation activity of traditional vinegar was more effective [31].

Many studies have described the relationship between total phenolic content and antioxidant activity. In previous reports, the DPPH scavenging activity of phenolic compounds were positively correlated with the amount of hydroxyl groups [45]. These phenolic compounds were found in rice grain fermented to rice vinegar. In comparisons between unpolished (or colored) rice and polished (or colorless) rice vinegar, the results indicated that the unpolished rice vinegars exhibited higher antioxidant activity due to the high amounts of total phenolic content. These findings were consistent with a previous study of dihydroferulic acid (DFA) and dihydrosinapic acid (DSA), ferulic acid and sinapic acid isolated from Japanese unpolished rice vinegar (Kurosu) and polished rice vinegar (Komesu), which were described as antioxidative compounds. These antioxidant compounds from Kurosu extract contained higher levels than in Komesu; therefore, Kurosu showed higher antioxidant activity. It has been suggested that unpolished rice vinegar is more advantageous than polished rice vinegar for antioxidative use [10]. Thus, these antioxidative compounds of rice vinegars were found in the rice grain. Particularly, colored rice (or pigmented rice), such as black glutinous and black fragrant rice have large contents of anthocyanin. Many researchers have reported the antioxidant properties of anthocyanin from pigmented rice. Therefore, colored rice has shown more antioxidant activity than white (or colorless) rice [12,13]. Consequently, these phenolic compounds influence the antioxidant activity of Thai rice vinegar products.

Furthermore, the influence of storing and aging on the DPPH radical scavenging activity of Zhenjiang aromatic vinegar (Chinese sticky rice vinegar) was investigated. Two years of vinegar storage provided higher antioxidant activity than fresh-made vinegar, but the total phenolic content was not significantly different. During storage, melanoidins (the brown polymer) were formed by the Maillard reaction, making the color of the vinegar become darker. The vinegar melanoidins were important for antioxidant activity [11].

4. Conclusions

Thai rice vinegars were produced from four types of Thai rice by the traditional method of solid-state fermentation. The alcohol fermentation step was developed by using enrichment of baker's dried yeast for inoculum preparation. Under the optimal conditions, the maximum of total acid productivity was detected in glutinous rice vinegar with a percentage yield of 6.77%, followed by black glutinous rice vinegar, black fragrant rice vinegar and polished rice vinegar with percentages of 5.44%, 4.10% and 3.83%, respectively. This result indicated that all samples of Thai rice were usable as an initial material for vinegar production. The pigment containing rice vinegars (glutinous rice- and black fragrant rice vinegar) contained more phenolic compounds than colorless vinegars (glutinous rice- and polished rice vinegar) This also indicated that the phenolic compounds contributed to the antioxidant activity in rice vinegars as the black glutinous rice vinegar exhibited the strongest antioxidant activity against both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals. The amounts of total phenolic compounds in Thai rice vinegars were not only related to their antioxidant activity but also provided encouraging anticancer activity on colon cancer cell lines. Moreover, the biological properties of Thai rice vinegars demonstrated that all Thai rice vinegars exhibited antibacterial activity against both Gram-positive and Gram-negative

bacteria tested. The antibacterial activity was related to the total acid and acetic acid contents of vinegars. As the produced rice vinegars contain effective biological activities, this fermentation process might be the alternative way for the utilization of traditional Thai rice. Furthermore, this study supports the information of the health benefit of Thai rice vinegars, leading to the advance stage of health benefit investigation.

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