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Research Article
Gamma-aminobutyric Acid, Total Anthocyanin Content and Antioxidant Activity of Vinegar Brewed from Germinated Pigmented Rice

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Abstract

Objective: The aim of this study was to produce healthy fermented vinegar from germinated pigmented rice. **Methodology:** The experiment was divided into four groups including germinated Hom Nil rice (purple rice), germinated Riceberry rice (purple rice), germinated Hom Mali Daeng rice (red rice) and control rice (ungerminated Riceberry rice). Vinegar was prepared using germinated pigmented rice as the raw material and was supplemented with red yeast rice, alpha-amylase and glucoamylase during saccharification, as well as *Saccharomyces cerevisiae* E1118 and *Acetobacter pasteurianum* TISTR 102 during alcohol and acetic acid fermentation. The functional and physicochemical properties of the vinegar were studied. **Results:** After 4 days of alcoholic fermentation, the alcohol content reached 8.55% for all rice powder samples, whereas the total soluble solids content decreased from 12.04-14.03 °Brix to 4 °Brix. During the acetic acid fermentation, there was little change in pH, although the acidity increased from 0.51-2.82%. The concentration of gamma-aminobutyric acid (GABA) in all samples ranged between 1.14 and 1.73 mg L^{-1} and the highest GABA content was found in the germinated Hom Nil rice. The germination process for all pigmented rice samples decreased the total anthocyanin content compared to the control samples. All vinegar samples had antioxidant activity, especially the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity in vinegar from germinated Riceberry rice and germinated Hom Mali Daeng rice that was relatively high compared to other samples. The sensory test revealed that samples of germinated Hom Mali Daeng rice exhibited a higher overall acceptance score than the other samples. **Conclusion:** This study indicated that brew-germinated pigmented rice vinegar could be used as a new product with antioxidant activity that was comparable to other vinegar products.

Key words: Germinated rice, pigmented rice vinegar, anthocyanin, GABA, antioxidant activity

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

For over 2000 years, vinegar has been used not only for acidic seasoning but also for preserving foods[1]. Several types of vinegars are produced worldwide. They have different raw materials, such as grapes, apples, wheat and rice. In some countries, vinegar is also used as a health drink[2]. Recent reports have suggested that vinegar provides some beneficial effects, such as digestive, appetite stimulation, antioxidant, exhaustion recovery and lipid lowering effects as well as regulation of blood pressure[3]. According to the latest data from the FAOSTAT[4], Thailand is one of the top ten rice producers in the world and a large portion of this production is harvested in the central region of Thailand. Every year, Thai rice production has an oversupply that leads to a drop in rice product prices[5]. Vinegar is generally an inexpensive product and its production only requires low-cost raw materials, such as rice[6]. In addition, there is a growing demand for rice vinegars, which are sold as a health food[7].

Rice vinegar, which is made from rice or rice wine is a traditional food in Asian countries. Lower in acidity than other vinegars, rice vinegar is not suitable for pickling or preserving foods. Vinegar fermentation from rice requires a saccharification step in addition to the alcohol fermentation and the oxidation of ethanol to acetic acid. The latter two steps are common in vinegar fermentation from materials that are rich in sugar, e.g., fruit juices. Rice vinegar is produced via a fermentation process that is carried out by several microorganisms including molds, yeasts, lactic acid bacteria and acetic acid bacteria[2]. These organisms produce not only acetic acid, but also various metabolic compounds that modify the taste and flavor of the vinegar.

Rice genotypes with either red, purple or black bran layers have been cultivated for a long time in Asia[8]. In pigmented rice, there are naturally occurring color substances that belong to a flavonoid group called anthocyanins. Positive health effects have been reported for the pigments present in the bran layer of rice. A commonly found anthocyanin in colored rice is acelylated procyanidin, which was reported to have free radical scavenging activity[9]. Germination of rice grains commonly produces gamma-aminobutyric acid (GABA), which is a free amino acid that has been shown to help relieve or prevent non-communicable diseases in human[10]. Several research studies have been conducted to explore alternative methods for producing a higher GABA yield. To obtain a higher GABA yield, sprouted rice was soaked in sodium glutamate[7]. Red yeast rice is a Chinese product of rice fermented using *Monascus purpureus*. Red yeast rice has been used in Chinese cuisine and medicinal foods to promote blood circulation for centuries. The use of red yeast rice in vinegars is prevalent in China. Recently, it was discovered that red yeast rice contains substances that are similar to prescription medications that lower cholesterol, such as of antihypercholesterolemic agents, including monacolin K and hypotensive agents of which GABA and antioxidants are also composed[11]. These compounds are very important for human health and are key components of health foods, also known as functional foods.

For this study, we brewed rice vinegar using germinated pigmented rice as the raw material and supplemented it with red yeast rice, alpha-amylase and glucoamylase. The objective of this study was to evaluate the changes in the GABA content, anthocyanin and antioxidant activity during the production process of rice vinegar. The quality characteristics of rice vinegar were also profiled during fermentation by measuring °Brix, pH, titratable acidity, color value and sensory analysis.

MATERIALS AND METHODS

Rice samples: The rice samples included three varieties of rice (*Oryza sativa* L.) including three Thai pigmented rice varieties: Hom Nil rice (purple rice), Riceberry rice (purple rice) and Hom Mali Daeng rice (red rice). All rice samples were harvested from Mahasarakham province, Thailand.

Germination of pigmented rice samples: The germination process was conducted according a previously described method used by Chen and Chen[7] with some modifications. Briefly, 500 g de-hulled rice grains were steeped in water at 7°C for 12 h. The water was drained off and the grains were then incubated in a plastic container and placed on eight pieces of cloth absorbing 150 mL of solution containing 1 g calcium chloride and 6 g sodium glutamate at 30°C for 4 days. After 1 mm of germination, the rice grains were dried at 50°C to maintain moisture content <11%. The dried grains were pulverized in a domestic grinder to obtain ground rice powder. Non-germinated rice (Riceberry rice) was used for comparison.

Preparation of red yeast rice: Preparation of red yeast rice was carried out by first soaking de-hulled rice in water at 30°C for 2 h. Water was drained off and 100 g of rice was put in a 500 mL Erlenmeyer flask and then was autoclaved at 15 psi and a temperature of 121°C for 20 min. After cooling, a 5 mL spore suspension from a one-week-old pre-cultured *Monascus purpureus* TISTR 3615 PDA slant was used for inoculation. The inoculated rice was incubated at 30°C for

15 days. The end-product was dried to a constant weight at 40°C to obtain dried red yeast rice, which was then smashed into pieces through a 40 mesh sieve and stored in the dark.

Preparation of an acetic acid bacteria culture: The acetic acid bacteria strain used in this study was a freeze-dried pure strain of *Acetobacter pasteurianum* TISTR 102 (TISTR, Thailand). This strain was propagated in glucose yeast extract broth (GYEB) and incubated at 30°C and 200 rpm for 3 days. The mass of 3% of the cultured was determined by measuring the optical density at 600 nm and then the bacteria were cultivated on a sterilized substrate containing 250 mL coconut water along with 0.25 g yeast extract and 7 mL of 95% ethanol in a 500 mL Erlenmeyer flask. The starter cultured was incubated under the same culture conditions for 4 days and it was used as the vinegar starter for acetic fermentation.

Acetic fermentation: Fermentation was conducted according to the previous method described by Chen and Chen[7], but some modifications were made. One hundred and twenty grams of germinated pigmented rice powder were added to 700 mL of water and added to 0.14 mL alpha-amylase (IU: 180,000 U mL^{-1}) in a 1,000 mL Erlenmeyer flask and sterilized at 121°C for 30 min. After cooling the samples to 60-70 and 40-50°C, 1.2 mL alpha-amylase and 0.57 mL glucoamylase (IU: 180,000 U mL^{-1}) were added to the sterilized mash, respectively. For alcohol fermentation, 0.7 g of dry yeast (*Saccharomyces cerevisiae* E1118) and 4.8 g of red yeast rice were added to the sterilized mash at 30°C. The mixture was incubated at room temperature for 4 days. When the alcoholic fermentation was completed and the alcohol content reached 8% (w/v), 200 ppm of potassium metabisulfite was added to stop fermentation and the samples were incubated for 2 h at room temperature. The mixture was filtered with cheese cloth and the supernatant was kept in wide mouth glass containers. Next, acetic acid fermentation in a liquid state was performed after inoculating the sample with 15% (v/v) of vinegar starter *A. pasteurianum* TISTR 102. The mouths of the glass jars were covered with cheese cloth and the broth was incubated at room temperature in a static state for 14 days. The vinegars that were obtained were pasteurized as a final treatment.

Analytical protocols

Determination of alcohol content and total soluble solids content of the rice wine: The alcohol content was determined using an ebulliometer. Total Soluble Solids (TSS) content was determined at 20°C with a refractometer in units of °Brix.

Determination of pH and titratable acidity of the brewed rice vinegar: Samples were centrifuged and the pH was measured at 20°C using a pH meter (Sartorius Docu-pH+Meter). The titratable acidity for the acetic acid was determined by titration to pH 8.2 with 0.1 N NaOH[12]. The results are expressed in g 100 mL^{-1} (or %) of acetic acid.

Color values of the brewed rice vinegar: The colors of brewed rice vinegar were measured objectively using Hunter's Lab color measurement instruments (The ColorFlex EZ's 45°/0° spectrophotometer, Hunter Lab, USA). The Hunter color values L*, a* and b* were measured.

Determination of total anthocyanin content of the brewed rice vinegar: The total anthocyanin content was determined with the pH-differential method[13]. Briefly, 20 µL of vinegar solution was transferred into a test tube for preparing two dilutions of the sample. One dilution contained 3 mL of potassium chloride buffer, pH 1.0 and the other dilution contained 3 mL of sodium acetate buffer, pH 4.5. These dilutions equilibrated for 15 min. The absorbance was measured for each dilution at 510 and 700 nm against a blank cell filled with distilled water. Absorbance readings were made against water blanks. The anthocyanin pigment concentration was calculated and expressed as cyanidin-3-glucoside equivalents using the following equation:

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg } L^{-1}) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

where, A = $(A_{520nm}-A_{700nm})$ pH 1.0-$(A_{520nm}-A_{700nm})$ pH 4.5, Molecular Weight (MW) is 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu), DF is dilution factor, l is path length in cm, ε is 26,900 molar extinction coefficient, in $L \times mol^{-1} \times cm^{-1}$ for cyd-3-glu and 10^3 is factor for conversion from gram to milligram.

Determination of GABA content in the rice powder and brewed rice vinegar: The GABA content measurement was carried out using the method of Phuapaiboon *et al.*[14] with some modifications. The germinated pigmented rice powder was extracted (2.5 g) with 70% (v/v) ethanol. The mixture was vigorously mixed for 1 min and then centrifuged at 10,000 rpm for 2 min. The supernatant was collected in a glass tube. The same volume of 70% ethanol solution was added to the precipitates as described above and the extraction was repeated twice. The collected supernatant (75 mL) was dried on a Büchi Syncore Polyvap (Büchi, Switzerland). The residues were dissolved in 5 mL of 70% ethanol solution and centrifuged at 10,000 rpm for 2 min. One milliliter of sample

extract or GABA standard solution (4-aminobutyric acid) was derivatized with 1 mL (1,000 μg mL^{-1}) of 9-fluorenylmethyl chloroformate (FMOC-Cl) diluted with borate buffer (pH 10) to the final volume of 5 mL and shaken for 30 sec, then incubated for 15 min at room temperature. The solution was filtered through 0.22 μm membrane filter. The filtered supernatant was analyzed by a HPLC (Waters 600 HPLC system) equipped with a Waters Symmetry reversed phase C18 column. The mobile phase A was 0.05% trifluoroacetic acid, the mobile phase B was methanol and the mobile phase C was acetonitrile. Ten microliters of each sample was injected and detected with a fluorescence detector (Jusco FP-920) at an excitation wavelength of 271 nm, an emission wavelength of 315 nm and a column temperature of 40°C. The vinegar was centrifuged at 10,000 rpm for 2 min and its GABA content was detected according to the method described above.

Determination of the antioxidant activity/capacity of brewed rice vinegar: Two methods, $DPPH^{\cdot}$ and $ABTS^{\cdot}$ based on the reaction with electron-donating or hydrogen-radicals ($H^{\cdot}$) producing compounds/antioxidants according to the following reaction were used:

$$R^{\cdot}+Aox\text{-}H \rightarrow RH+Aox^{\cdot}$$

Where:

$R^{\cdot}$ = $DPPH^{\cdot}$, $ABTS^{\cdot+}$ or other reactive radical
Aox-H = Ph-OH, trolox, vitamin C, etc.
Ph = Polyphenolic compound

DPPH radical scavenging assay: The free-radical scavenging capacity of each vinegar sample was evaluated according to the procedures of Maisuthisakul and Gordon[15] with some modifications. Briefly, 200 and 100 μL of methanol were added to blank and control wells, respectively. One hundred microliters of vitamin C (standard) and the samples of vinegar were added to the appropriate wells. Then, freshly prepared 0.25 mM DPPH in methanol solution (100 μL) was mixed with control, standard and sample wells. The plate was gently shaken for 5 sec. After the solution was incubated at 37°C for 30 min in a dark room, the absorbance of the reaction mixture was read at 520 nm with the lid on the plate in a model 680 microplate reader (spectrophotometer). The antioxidant activity was calculated with the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{520}\ \text{control}-A_{520}\ \text{test sample}}{A_{520}\ \text{control}} \times 100$$

The inhibition percentage of the absorbance of DPPH was plotted against each quantity of vinegar to obtain a regression line. Vitamin C was used as a standard to convert the inhibition capability of vinegar to the vitamin C equivalent of antioxidant activity. The ratio of the slopes of the regression lines of the extract solution and the vitamin C solution were defined as the vitamin C equivalent antioxidant capacity. Then, it was converted to mM vitamin C equivalent antioxidant capacity (mM VCEAC).

ABTS radical scavenging assay: For the ABTS assay, the procedure followed the method of Seeram *et al.*[16] with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in 8 mL ABTS solution and 12 mL potassium persulfate solution and then incubating the solutions for 18 h at room temperature in the dark. The solution was then diluted by mixing 5 mL $ABTS^{\cdot+}$ solution with 10 mL methanol before use. Then $ABTS^{\cdot+}$ solutions (50 μL) were added to samples of vinegar (200 μL). These solutions were gently mixed and incubated in the dark for 30 min at room temperature. Then the absorbance values of the resulting solutions were measured at 750 nm in a microplate reader spectrophotometer. Trolox was used as a reference compound and a standard calibration curve was prepared using trolox in a range of 50-300 mM. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) values.

Sensory analysis: The affective test to evaluate appearance, color, flavor, taste and overall acceptance of the brewed germinated pigmented rice vinegar was performed by 32 panelists using a 5-point hedonic scale. A structure scale was used to score all the attributes, with 1 representing very bad, 3 representing normal and 5 representing very good. Coded samples identified by three-digit random numbers were presented to panelists in random order.

Statistical analysis: The experiment was conducted in a completely randomized design with three replications. The results were submitted to analysis of variance (ANOVA) to determine significant differences ($p<0.05$) between different types of treatments using SPSS 13 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Fermentation conditions and parameters of the rice wine: For fermentation, the powders of ungerminated Riceberry rice (control), germinated Hom Nil rice, germinated Hom Mali Daeng rice and germinated Riceberry were separately added to water and sterilized. The initial sugar concentrations of the

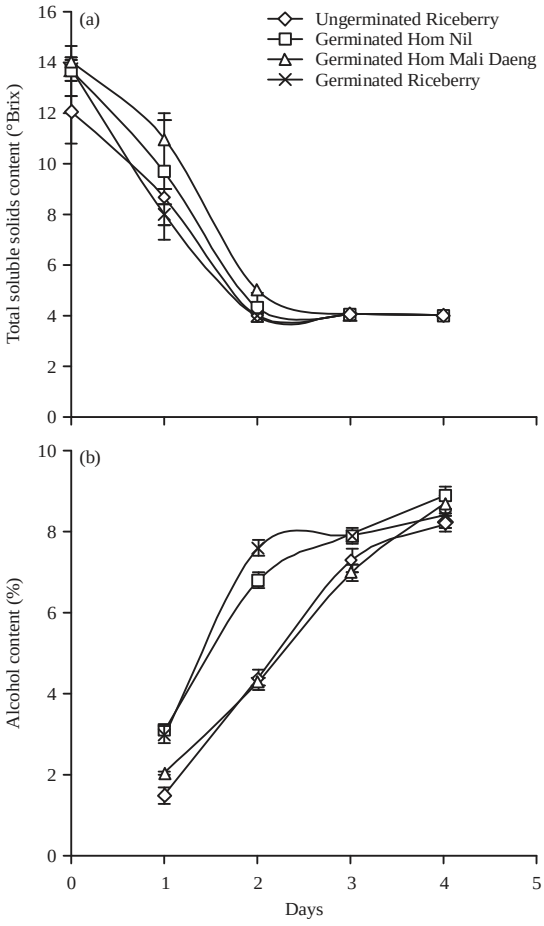

Fig. 1(a-b): Change in (a) Total soluble solids content and (b) Alcohol content during alcoholic fermentation of pigmented rice

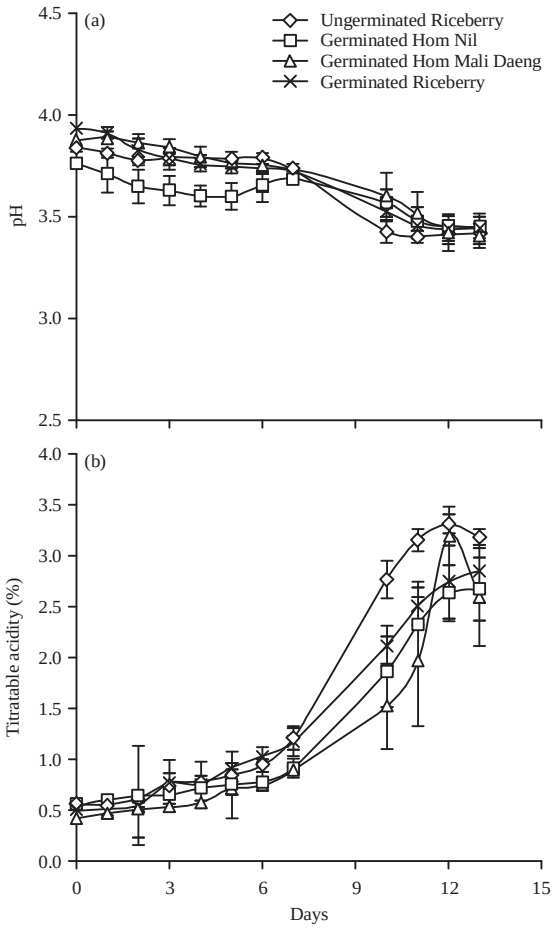

Fig. 2(a-b): Change in (a) pH and (b) Titratable acidity during acetic acid fermentation of pigmented rice

mashes were produced by liquefying alpha-amylase and saccharifying glucoamylase. The fermentation curves of the mashes are shown in Fig. 1. During 4 days of fermentation, the total soluble solids content decreased from 12.04-14.03°Brix to 4°Brix in the samples (Fig. 1a) and the alcohol content increased from 1.5-3.1 to 8.2-8.9% in the samples (Fig. 1b). The fermentation was stopped after 4 days when the alcohol content was higher than 8%, which is the alcohol content of common rice wines that produce acetic acid during fermentation with acetic acid bacteria[17]. Total soluble solids decreased most sharply in the germinated Riceberry rice sample from 13.73-4 °Brix during the first 2 days of fermentation compared to other samples. The germinated Riceberry rice sample had the highest alcohol content after 2 days of fermentation (7.6%), whereas, the germinated Hom Nil rice sample had the highest alcohol content after 4 days of fermentation (8.9%) compared to the other samples.

pH and titratable acidity of the brewed rice vinegar: The rice wine used as a substrate for acetic fermentation had changes in pH and titratable acidity, which are shown in Fig. 2. The average pH value of all samples decreased from 3.85-3.43 over 13 days of acetic fermentation (Fig. 2a). The acidity of all samples increased gradually during acetic fermentation (Fig. 2b). After 13 days, the average value of acidity of all samples reached 2.82%. Sample ungerminated Riceberry rice showed the highest acidity at 13 days of acetic fermentation (3.18%) compared to other samples, which was not significant. The final acidity achieved in the present study was lower than the acidity achieved in rice vinegar produced with a submerged fermentation process using Acetator® (6.85%)[18]. However, production of rice vinegar by submerged fermentation is used in processes with generators (the rapid process). Bacteria are always submerged in liquid to ferment, where they multiply and oxidize the alcohol mixture into vinegar. The acidity differences in various vinegars could

Table 1: Hunter's color value of the brewed rice vinegar (Mean±SD)

Brewed rice vinegar type	L*	a*	b*
Ungerminated Riceberry	21.20±0.54[a]	7.34±2.90[c]	8.65±1.66[ab]
Germinated Hom Nil	21.37±0.95[a]	3.53±2.05[ab]	9.88±0.31[b]
Germinated Hom Mali Daeng	29.29±4.56[b]	0.18±0.36[a]	7.03±0.68[a]
Germinated Riceberry	26.98±1.36[b]	5.22±0.69[bc]	9.70±0.26[b]

Different letters within the columns indicate significant difference (p<0.05)

Table 2: Total anthocyanin content of the brewed rice vinegar (Mean±SD)

Brewed rice vinegar type	Values (mg L^{-1})
Ungerminated Riceberry	1.62±0.24[b]
Germinated Hom Nil	0.13±0.08[a]
Germinated Hom Mali Daeng	0.29±0.13[a]
Germinated Riceberry	0.68±0.49[a]

Different letters within the columns indicate significant difference (p<0.05)

Table 3: GABA content of the rice powder and the brewed rice vinegar (Mean±SD)

Samples	Rice powder (mg/100 g DW)	Brewed rice vinegar (mg L^{-1})
Ungerminated Riceberry	0.24±0.03[a]	1.59±0.14[bc]
Germinated Hom Nil	1.51±0.50[ab]	1.73±0.20[c]
Germinated Hom Mali Daeng	3.15±1.11[bc]	1.21±0.06[ab]
Germinated Riceberry	3.91±0.71[c]	1.14±0.14[a]
Red yeast rice	0.93±0.50[a]	-

Different letters within the columns indicate significant difference (p<0.05), DW: Dry weight

be attributed to variations in the raw materials, the amount of acetic acid bacteria added and the fermentation time as well as dilution.

Color of the brewed rice vinegar: Color is an important quality attribute of a product like vinegar. The L* is the lightness-darkness value, a* is the red-green axis value and b* is the yellow-blue axis value. The color of brewed rice vinegar was measured and compared among the four samples (Table 1). The colors obtained from the ungerminated Riceberry rice sample and germinated Hom Nil rice were different (p<0.05) in terms of the L* value characteristics compared to the germinated Hom Mali Daeng rice and germinated Riceberry rice samples. The ungerminated and germinated Riceberry rice samples showed a higher a* value (redness) (p<0.05) than germinated Hom Mali Daeng rice samples. These differences occurred because Riceberry rice contains anthocyanin pigments, such as cyanidin-3-glucoside and peonidin-3-glucoside, in the bran layer[19]. The b* value (yellowness) of all samples ranged between 7.03 and 9.88 and the germinated Hom Nil rice and germinated Riceberry rice samples had a higher b* value (p<0.05) than germinated Hom Mali Daeng rice sample.

Total anthocyanin content of the brewed rice vinegar: The total anthocyanin content of all samples of this study ranged from 0.13-1.62 mg L^{-1} (Table 2). The ungerminated Riceberry rice samples exhibited the highest (p<0.05) total anthocyanin content with 1.62 mg L^{-1} in this study, whereas all samples of germinated pigmented rice had a lower total anthocyanin content. In this study, germination had an effect on the total anthocyanin content for all pigmented rice varieties. These effects occurred due to the loss of anthocyanin during the soaking stage, which was similar to the effects reported by Sutharut and Sudarat[20]. However, this study showed that Riceberry rice contained the highest amount of anthocyanin among the three varieties. According to Jittorntrum *et al.*[21], Riceberry rice has abundant anthocyanin and the rice bran extracts of Riceberry rice contained major and minor proanthocyanidin in rice as well as cyanidin and peonidin in amounts of 150.81 and 66.76 mg/100 g, respectively. These compounds are pigmented and have unique colors, such as purple, red or black. Black and red rice contain 2 main anthocyanin compounds, cyanidin-3-glucoside and peonidin-3-glucoside and cyanidin-3-glucoside comprises approximately 93% of the measured anthocyanin[20]. Takeshita *et al.*[22] studied anthocyanin content in alcoholic beverages made with uncooked unpolished black rice and reported that the anthocyanin content of black rice alcoholic beverages was 94.6 mg L^{-1}, which was higher than the value recorded in this study. This difference might have occurred because a part of the anthocyanin pigment might have been denatured during the cooking process. Moreover, anthocyanin is easily destroyed by an unsuitable pH and aerobic state. From the results of Table 2, brewed rice vinegar made from germinated Hom Mali Daeng rice (red rice) contained anthocyanin (0.29 mg L^{-1}), whereas, Takeshita *et al.*[22] reported that alcoholic beverages made from red rice did not appear to contain anthocyanin.

GABA content of the rice powder and brewed rice vinegar: After soaking the samples and incubating the samples, the GABA content in germinated pigmented rice powder was generally higher than that in ungerminated pigmented rice powder (Table 3). The results indicated that the storage protein in rice grains was decomposed and supplied to the growing part of the seedlings and within this process, the glutamate decarboxylase enzyme was activated, which converted glutamic acid to GABA. In agreement with the results of Karladee and Suriyong[23], the GABA content in 21 rice varieties of brown rice (11 landraces purple rice and 10 modern white varieties) was significantly increased during soaking. This germination process indicates the importance of pigmented rice cultivars for adding nutritional value to functional food products. The concentration of GABA was 0.24 mg/100 g dry weight in ungerminated Riceberry rice

powder. After germination, the amount of GABA significantly increased in Riceberry rice powder. The germinated Riceberry rice powder exhibited higher GABA content than did the ungerminated Riceberry rice powder and the germinated Hom Nil rice powder ($p<0.05$). From the results in Table 3, the level of GABA in the red yeast rice sample was somewhat lower than the germinated Riceberry rice powder and germinated Hom Mali Daeng rice powder samples ($p<0.05$). This result contrasted a previous study that demonstrated efficient methods to increase GABA in rice grains and red yeast rice using a fermentation method, which produced a higher GABA concentration compared to germinated rice of most rice cultivars[24]. However, different species of *Monascus* sp. were used in various rice cultivars as substrates for GABA production and fermentation time may have had an effect on GABA content in red yeast rice.

In this study, we produced brewed vinegar using two-stage fermentation. In the first stage, starch was hydrolyzed into fermentable sugar with saccharification enzymes, while fermentable sugar was the fermented into ethanol by yeasts. In addition, red yeast rice powder was added into fermentation mash to improve the GABA content. In the second stage, ethanol in the fermentation mash was converted into acetic acid by acetic acid bacteria. The results in Table 3 show that the GABA content of all brewed vinegar samples ranged between 1.14 and 1.73 mg L^{-1} with an average of 1.42 mg L^{-1}. The germinated Hom Nil rice showed a slightly higher GABA content than did germinated Hom Mali Daeng rice and germinated Riceberry rice samples ($p<0.05$). In contrast, the germinated Riceberry rice sample showed a slightly lower GABA content than did the ungerminated Riceberry rice sample ($p<0.05$). Liu *et al.*[25] reported the GABA content in rice wine using near infrared spectroscopy with an optical fiber probe and predicted the content to be between 157.6696-317.5813 mg L^{-1}. In addition, Tamaruay *et al.*[26] found that fermented-germinated brown rice juice and fermented black rice juice had GABA content so 2.25 ± 0.17 and 0.97 ± 0.07 mg g^{-1}, respectively. However, the results of this study showed that the average amount of GABA content in the four types of brewed rice vinegar were lower than a previous study. This discrepancy is probably due to sterilizing the samples at 121 °C for 30 min of cooking, which likely resulted in a loss of GABA in the germinated pigmented rice. Cooking processes can cause considerable losses in vitamins and minerals. According to Tiansawang *et al.*[27], domestic traditional cooking processes, such as boiling, steaming, microwave cooking and open pan roasting were found to decrease GABA content in germinated grains and steaming was found to reduce GABA content the least in black beans and soybeans, whereas, microwave cooking led to the smallest loss of GABA content in mung bean and sesame. Moreover, GABA content in this study did not correspond with the study of Chen and Chen[7]. The researchers reported that the GABA content in finished rice vinegar was 100 mg L^{-1}, which was produced from 500 g smashed sprouted rice, 4 g red yeast rice, 1.5 g raw starter complex, 1.6 mL glucoamylase preparation, 5 g sodium glutamate and 335 mL water fermented at pH 6 and 28 °C for 8 days.

Antioxidant activity of the brewed rice vinegar: The content of the antioxidant activity values for brewed rice vinegar are presented in Table 4. Vitamin C and trolox were used as standards for the calibration of the DPPH and ABTS methods, respectively, for the assessment of antioxidant activity. The DPPH method was the most reactive one for phenolic compounds and yielded higher values than the ABTS method. The higher reactivity of the DPPH reagent with phenolic compounds is the most important factor. In the DPPH method, the average antioxidant activity value was 11.19 mM VCEAC (or 46.18%) in all samples. The highest antioxidant activity was found in the germinated Hom Nil rice and germinated Hom Mali Daeng rice samples (16.85 mM VCEAC or 51.29% and 14.46 mM VCEAC or 49.16%, respectively). The lowest value, which was less than half of the highest value was found in the ungerminated Riceberry rice sample (5.05 mM VCEAC or 40.53%). From this result, DPPH activity in the germinated pigmented rice sample appeared to be higher than in the ungerminated pigmented rice sample. This result likely because the germination process affected the phenolic content of germinated grain. In agreement with Tiansawang *et al.*[27], the DPPH activity in soybean and sesame increased during germination. In contrast, the same

Table 4: Antioxidant activity of the brewed rice vinegar (Mean ± SD)

	DPPH		ABTS	
Brewed rice vinegar type	Scavenging activity (%)	mM VCEAC	μg TEAC mL^{-1}	μM TEAC
Ungerminated Riceberry	40.53 ± 4.74^{a}	5.05 ± 4.94^{a}	8.19 ± 1.46^{a}	34.81 ± 6.23^{a}
Germinated Hom Nil	51.29 ± 3.03^{c}	16.85 ± 3.40^{c}	9.51 ± 2.64^{a}	40.44 ± 11.24^{a}
Germinated Hom Mali Daeng	49.16 ± 2.13^{bc}	14.46 ± 2.39^{bc}	9.37 ± 1.80^{a}	39.81 ± 7.60^{a}
Germinated Riceberry	43.75 ± 4.60^{ab}	8.38 ± 5.16^{ab}	9.81 ± 0.87^{a}	41.69 ± 3.73^{a}

Different letters within the columns indicate significant difference ($p<0.05$)

Table 5: Sensory evaluation of the brewed rice vinegar (Mean±SD)

Brew rice vinegar type	Appearance	Color	Flavor	Taste	Overall acceptance
Ungerminated Riceberry	2.65±1.40^{a}	2.37±0.97^{a}	2.53±1.04^{a}	3.43±1.16^{a}	2.78±0.94^{a}
Germinated Hom Nil	3.46±0.98^{b}	3.15±0.80^{b}	2.96±0.93^{a}	2.96±0.99^{a}	3.18±0.73^{ab}
Germinated Hom Mali Daeng	4.31±0.85^{c}	3.75±1.16^{c}	3.09±0.99^{a}	3.09±0.96^{a}	3.31±0.89^{b}
Germinated Riceberry	3.15±1.05^{b}	2.75±1.01^{ab}	2.96±1.23^{a}	3.00±0.98^{a}	3.03±0.89^{ab}

Different letters within the columns indicate significant difference ($p<0.05$)

researchers also found that the DPPH activity in mung bean slightly decreased and total phenolic content showed no significant changes during germination. This outcome indicated that genetics can be an important factor for phenolic compound synthesis during germination. Takeshita *et al.*[22] found that DPPH activity ranged from approximately 1.2 to approximately 1.8 mM trolox equivalent units for alcoholic beverages made from black rice, red rice and wild rice. These DPPH activity values were lower than the values measured for this study. This discrepancy could be caused by methodological differences. Singkong[17] reported that the antioxidants of red wine made with black jasmine rice produced an average DHHP activity of 86.21%, which was higher than that in this study. This result agreed with results reported in the literature in which the antioxidant activity of blueberry wine was higher than that of the blueberry extract and blueberry vinegar[28]. In the ABTS method, the average antioxidant activity of all samples of brewed rice vinegar was 39.19 μM TEAC (or 9.22 μg TEAC mL^{-1}). There were no significant differences ($p>0.05$) between the ungerminated pigmented rice and germinated pigmented rice samples. However, there was a tendency for ABTS activity of all samples of germinated pigmented rice to be higher compared to the ungerminated Riceberry rice, which was not statistically significant.

Sensory evaluation of the brewed rice vinegar: For the brewed rice vinegar, various organoleptic attributes were evaluated, including the appearance, color, flavor, taste and overall acceptance (Table 5). Sensory evaluation was performed with brewed rice vinegar containing different varieties of pigmented rice. The germinated Hom Mali Daeng rice sample showed the best results for appearance (4.13), color (3.75) and overall acceptance (3.31). In flavor and taste, there were no significant differences among the samples. Brewed rice vinegar made from germinated Hom Mali Daeng rice had the highest acceptance, which might be a result of the panelists' perception of appearance and color. In addition, this sample had the highest values for L* (29.29) and the lowest values for a* (0.18) and b* (7.03), which might have affected the overall acceptance score. The overall acceptability of brewed rice vinegar made from ungerminated and germinated pigmented rice ranged between 2.78 and 3.31, which indicated moderated acceptance.

CONCLUSION

This study was performed to investigate the functional and physicochemical properties of brewed vinegar produced with pigmented rice. During the acetic acid fermentation, there was little change in pH, yet the acidity increased from 0.51-2.82%. Total anthocyanin content of germinated pigmented rice influenced the germination process. Moreover, the sterilization process decreased GABA content in germinated pigmented rice. However, vinegar produced by fermentation of germinated pigmented rice showed that antioxidant activity was comparable to other vinegars using the DPPH and ABTS methods. The results of this study indicated that brewed germinated pigmented rice vinegar could be used as a new product with antioxidant activity that is comparable to other vinegar products.

SIGNIFICANCE STATEMENT

Production of vinegar via fermentation of germinated pigmented rice represents a promising approach to developing functional foods. Vinegar can be produced through different methods and with various raw materials. Rice vinegar is made by fermenting the sugars in rice into alcohol and then into acid. Rice vinegar is less acidic. It is commonly used in salad dressings and is also currently used as a health drink. This study determined the gamma-aminobutyric acid (GABA) content in vinegar made with germinated rice grains as the raw material and supplementing the grains with red yeast rice. Anthocyanin is one of the antioxidant compounds that is produced in vinegar made with Thai pigmented rice. The GABA and antioxidants are also found in vinegar. These compounds are very important for human health. Therefore, this vinegar is a promising new health food.

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