Production of vinegar from organic broken rice noodles

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Abstract. Organic broken rice noodles (OBRN) are generally considered as waste material from noodle processing factories and are of low value. The purpose of this research study was to increase the value of OBRN by converting it to fermented vinegar. In the first step, the hydrolysis methods of OBRN were studied. The conversion of starch into reducing sugar using acid hydrolysis, mold inoculation, and rice cake starter (loog-pang) were compared. For acid hydrolysis, 0.6 M sulfuric or hydrochloric acid at 80, 100, and 121 °C was used. For biological hydrolysis, steamed OBRN was soaked in water at a ratio of 1:2 for 1 h, then mixed with 0.04 % (w/w) koji or rice cake starter and left to ferment. The latter methods yielded the highest glucose of 167.66 and 178.94 g/L, respectively, when 0.2 % of starters were inoculated for 3 days. In a second step, OBRN wine was made from a mixture of hydrolyzed OBRN liquid and 20 °Brix of pineapple juice at a 1:1 ratio and subsequently fermented for 7 days. The alcohol produced from OBRN showed the highest concentration of 10.05 % (v/v) at 7 days of incubation. Lastly, wine was prepared at various alcohol concentrations of 4-6 % (v/v) and at a pH of 5.5 for further vinegar fermentation using Acetobacter pasteurianus TISTR 102, by shaking at 150 rpm for 7 days. The result showed that the appropriate alcohol concentration for producing vinegar was 4 % (v/v) and the OBRN vinegar exhibited clear, light yellow color, and had total acidity content of 3.52 % (v/v) and a pH of 3.28 after 4 days of incubation.

1. Introduction
Vinegar is produced from raw materials containing sugar or starch by submerged culture. The production of vinegar typically involves two steps of fermentation. First, the sugars in the raw material are converted to alcohol using yeast, such as Saccharomyces cerevisiae, Saccharomyces fragilis, and Kluyveromyces fragilis, etc… The resulting alcohol is further oxidized to acetic acid by acetic acid bacteria such as Acetobacter pasteurianus and Gluconobacter oxydans, etc… in a second step [1]. Vinegar is used in food preparation including as a preservative for vegetables, fish, and meat, as well as in the preparation of mayonnaise, salad dressings, mustard, and other food condiments [2-3]. In addition, vinegar has been proven to have medicinal advantages by virtue of its physiological effects.
such as regulating blood glucose, blood pressure, aiding digestion, stimulating the appetite, promoting calcium absorption, and promoting recovery from exhaustion [4-6]. According to Thai Industrial Standards, vinegar is defined as a sour solution that must contain not less than 4 g of acetic acid per 100 cm³ and be produced from alcohol and successive acetic fermentation from sugary and starchy substrates.

In general, raw materials used for the production of fermented vinegar are fruits such as grapes, apples, plums, coconuts, and pineapples, etc... Carbohydrate ingredients including rice malt starch and various other agricultural materials are also used [7]. The conversion of starch into sugar can also be carried out by hydrolysis of food processing by-products such as broken noodles, with acid or mold or using rice-cake starter (loog-pang).

Thailand is one of the world leaders for food-product export; consequently, a large quantity of by-products from food processing plants, such as fruit and vegetables, rice, fat, and oil, are available [8]. These by-products cause a major disposal problem for the industry. Rice noodles are one of the main export products from Thailand with a value of about 555 million Thai baht in 2018 [9]. Organic food products including organic rice noodles are gaining popularity due to consumers’ health consciousness. The broken noodles of such products are considered as a by-product which results from the rolling and cutting process. Most of these broken rice noodles are often used as animal feed, fertilizers, or treated as waste, all of which have low value [10]. Therefore, if the waste or by-product from the rice processing plant can be used as a raw material for the production of other value-added products, including fermented vinegar, it would generate a higher value product.

Rice-cake starter is a traditional starter used to produce alcoholic beverages or indigenous food products in many Asian countries. These traditional starters have various names, such as marcha or murcha in India, ragi in Indonesia, bubod in the Philippines, Chinese yeast in Taiwan, nuruk in Korea [11], banh men in Vietnam, koji in Japan, ragi tapai in Malaysia, and loog-pang in Thailand [12]. Microorganisms in loog-pang include mold, bacteria, and yeast. Most studies found Amylomyces rouxii and Saccharomycopsis fibuligera are common mold and yeast, respectively, in loog-pang [13]. This research study aims at employing the by-product from the organic rice noodle processing as a substrate for vinegar fermentation. The suitable methods of hydrolysis and alcohol concentration were investigated, as well as some of the properties of the final product.

2. Material and methods

2.1. Materials
Organic broken rice noodles were obtained from Foodle Noodle Co., Ltd., Thailand. The rice-cake starter was purchased from a market in Nan Province, Thailand. The mold, Amylomyces rouxii TISTR 3182 used for the koji starter [14], and Acetobacter pasteurianus TISTR 102, used for acetic acid fermentation, were purchased from the Culture Collection Center, Thailand Institute of Scientific and Technological Research, Thailand.

2.2. Starch hydrolysis
Acid hydrolysis was performed using HCl or H₂SO₄ at 0.6 M. OBRN in acid solutions was submitted to a thermal treatment at different temperatures (80, 100, and 121 °C) for 15, 30, and 45 min. The samples were prepared in 250 ml flasks, using 10 g of OBRN and 90 ml of the acid solutions. The hydrolysis of OBRN using rice-cake starter and koji was started by soaking 100 g of OBRN in water at a ratio of 1:2 for 1 h, and steaming for 15 minutes, followed by mixing with various ratios of rice-cake starter and koji at 0, 0.1, 0.2, 0.3, and 0.4 % (w/w), and finally incubated at 30 °C for 3 days. Reducing sugar in the fermentation supernatant after centrifugation was determined by the dinitrosalicylic acid method (DNS method) [15].
2.3. Alcohol fermentation
Alcohol fermentation was carried out by adding 20 °Brix of pineapple juice into the liquid obtained from starch hydrolysis, using rice-cake starter at a ratio of 1:1 and incubated at 30 °C for 7 days. The samples were collected every day for physical and chemical analysis as stated in 2.5.

2.4. Acetic acid fermentation
The alcohol-containing liquid was adjusted to alcohol concentrations of 4, 5 and 6 % (v/v), with pH of 5.5, and further inoculated with 5 % of Acetobacter pasteurianus TISTR 102, and then shaking was carried out at 150 rpm, at 30 °C for 7 days. The samples were collected every day and subjected to analysis, as follows.

2.5. Physical and chemical analysis
Reducing sugar in the fermentation supernatant after centrifugation was determined by the DNS [15]. The pH was measured according to AOAC 2000 [16] by using a pH meter. Total acidity content in the vinegar was analyzed by titration with 0.1 M NaOH until a pH end-point of 8.4, and the acidity was calculated as acetic acid in vinegar according to AOAC 2000 [16]. The alcohol content was determined by an alcohol vapor sensor from Vernier Software & Technology, USA. The color of the vinegar was measured as L*, a* and b* using a Hunter lab.

3. Result and discussions

3.1. Starch hydrolysis between chemical and biological treatment
Figure 1 shows the reducing sugar concentration obtained from OBRN hydrolysis using HCl and H2SO4. The optimum conditions were 0.6 M HCl, with a reaction time of 30 min at 121 °C, which resulted in 118.80 g/L (92.64 % of theoretical yield) of reducing sugar. Acid hydrolysis was applied to digest various organic polysaccharides, in order to release the sugar molecules. Woiciechowki et al. (2002) [17] reported that when using 1 % hydrochloric acid to digest cassava bagasse, it was almost completely converted to glucose under optimum conditions (100 °C, for 30 min). Such conditions represented 94.5 % of recovery of reducing sugar from the starch available in cassava bagasse. Hot acid pre-treatment was also successful in Napier grass straw, where 0.7 % HCl was applied at 105 °C for 1 h [18].

![Figure 1. Reducing sugar obtained from the hydrolysis of OBRN by sulfuric acid and hydrochloric acid at 80, 100 and 121 °C for 15, 30 and 45 minutes.](image-url)
Reducing sugar obtained from the hydrolysis of OBRN by rice-cake starter and koji at different percentages of inoculum are shown in Figure 2. The optimum concentration of rice-cake starter or koji was 0.2 % (w/w) with the highest amount of reducing sugar obtained at 178.95 ±0.05 g/L (45.71% of theoretical yield) and 167.67 ±0.05 g/L (42.83 % of theoretical yield), respectively, at 3 days of cultivation. Molds in rice-cake starter were usually Rhizopus sp., Mucor sp., Amylomyces sp. and Aspergillus sp., and mold of koji can produce amylases and these enzymes hydrolyze starch into sugars [19-21].

![Figure 2](image_url)

**Figure 2.** Reducing sugar obtained from the hydrolysis of OBRN using rice cake starter or at various concentration.

The study showed that the process yields of recovered reducing sugar from OBRN hydrolysis by acid, rice-cake starter, and koji were 92.64 %, 45.71 %, and 42.83 %, respectively. Although starch hydrolysis by acid was the most efficient method concerning the yield based on the reducing sugar recovered from the OBRN, high process temperature during hydrolysis caused toxic compounds which might produce derivatives of furfur (2-furaldehyde and 5-hydroxymethyl-2-furaldehyde) [17]. Therefore, starch hydrolysis using rice cake starter was used for further study as it would be safe and easy to perform by the noodle processing plant.

### 3.2. Fermentation to alcohol

At the beginning of fermentation, the sugar concentration of samples was adjusted to 20 °Brix. The results of total soluble solid, alcohol content, pH, total acidity, and reducing sugar during alcoholic fermentation are presented in Table 1. The sugar level gradually declined inversely to the incrementation of alcohol. At the end of fermentation, 7 days, the samples contained 10.05±0.05 % (v/v) alcohol. It has been shown that yeasts in rice cake starter such as *Saccharomyces cerevisiae* mainly consumed sugar in the alcohol fermentation [22]. The titratable acidity of samples during the fermentation increased slightly from 0.32 to 1.08 %, and pH changed slightly. This increase in acidity provided optimal growth conditions to initiate acetification. The alteration in pH attributed to the accumulation of acetic acid and other organic acids such as propionic and tartaric acid, which are important for the development of flavor and aroma [23]. Adebayo-Oyetoro *et al.* (2017) [24] studied the production of vinegar from mango and found that the pH increased slightly from 3.98 to 4.02 and the total acidity increased from 0.02 to 4.02 % after 30 days of fermentation.
Table 1. Some chemical properties of organic broken rice noodles fermented by rice cake starter for 7 days.

| Time (Days) | Total soluble solid (°Brix) | Alcohol (%) | pH | Total acidity (%) |
|-------------|----------------------------|-------------|----|------------------|
| 0           | 21.33±0.15ᵇ                 | 4.43±0.01ᵉ  | 3.54±0.02ᶜᵈ | 0.32±0.03ᶠ       |
| 1           | 21.63±0.12ᵃ                 | 3.84±0.01ᵍ  | 3.54±0.02ᶜᵈ | 0.62±0.03ᵉ       |
| 2           | 20.93±0.12ᶜ                 | 3.66±0.01ʰ  | 3.52±0.02ᵈᵉ | 0.88±0.03ᵈ       |
| 3           | 18.27±0.12ᵈ                 | 4.27±0.01ᶠ  | 3.51±0.01ᵉ  | 0.88±0.03ᵈ       |
| 4           | 15.13±0.12ᵉ                 | 6.08±0.01ᵈᵉ | 3.55±0.01ᶜ  | 0.94±0.03ᵃ       |
| 5           | 10.17±0.06ᶠ                 | 7.50±0.01ᶜ  | 3.60±0.01ᵇ  | 1.00±0.03ᵇᶜ     |
| 6           | 9.17±0.06ᵍ                  | 8.04±0.00ᵇ  | 3.59±0.02ᵇ  | 1.04±0.03ᵇ       |
| 7           | 8.11±0.01ʰ                  | 10.05±0.05ᵃ | 3.66±0.02ᵃ  | 1.08±0.03ᵃ       |

Data represent the average of triplicate analysis,ᵃᵇ different letters in the same column are significantly different (p<0.05)

3.3. Acetic acid fermentation

The effects of different alcohol concentrations on acetic acid production from OBRN through vinegar fermentation with *Acetobacter pasteurianus* TISTR 102 are shown in Figure 3. The total titratable acidity calculated as acetic acid in all samples increased with fermentation time. After 2-4 days of fermentation, the acidity remained constant. The higher the alcohol concentration, the lower the acid concentration obtained. The initial alcohol content at 4 % (v/v) generated the highest percentage of acidity at 3.52±0.02 %, compared to those of 5 and 6 %, which resulted in 2.92±0.03 and 2.02±0.03 % (v/v), respectively. Ory *et al.* (2002) [25] studied the influence of ethanol concentration in vinegar fermentation and found that the optimal concentration for vinegar fermentation was 3.55-4.7 % (v/v). It has been proven that when ethanol concentration is over 4 % (v/v), it is very unlikely that any growth of bacteria will be seen [26]. In addition, the lag phase increases proportionally with the ethanol concentration. Therefore, the concentration of ethanol was adjusted to 4 % (v/v) for further study.

![Figure 3](image-url)
3.4. Physical and chemical analysis
Table 2 shows some properties of the final product of fermented vinegar. The vinegar produced had a total soluble solid of 7.20±0.02 °Brix, pH of 3.28±0.02 and 3.52±0.02 % total acidity (acetic acid). This fell below the 4 % acetic acid level allowed in the vinegar according to Thai industrial standards, as well as AOAC [16]. This could be attributed to an interruption of the air supply during the oxidation process, as acetic acid bacteria are reported to be very susceptible to air interruption during oxidative processes [27]. Therefore, in order to increase the acetic acid concentration, it is suggested that the oxygen concentration should be increased by aeration during acetic fermentation in the OBRN vinegar.

The alcohol content continued to decrease with time from 4.00 % to about 0.24 % at day 4, and the vinegar was clear and has a light-yellow color (39.89±0.83 of *L).

Table 2. Some properties of the vinegar obtained from the fermentation of OBRN.

| Parameters                  | Values               |
|-----------------------------|----------------------|
| Total soluble solids (°Brix)| 7.20±0.02            |
| Titratable acidity (%)      | 3.52±0.02            |
| pH                          | 3.28±0.02            |
| Alcohol content (%v/v)      | 0.28±0.04            |
| L*                          | 39.89±0.83           |
| a*                         | 1.65±0.24            |
| b*                         | 0.28±0.06            |
| h                          | 9.72±0.98            |

*Values are means ±SD of triplicate determinations.

4. Conclusion
This research study provides evidence showing that the use of organic broken rice noodles in alcohol fermentation by rice-cake starter (loog-pang) and acetic acid, using Acetobacter pasteurianus TISTR 102, is possible. The vinegar produced from organic broken rice noodles also has acceptable properties and quality. It is also implied that broken rice noodles which are conventionally regarded as waste in noodle processing can be converted into a value-added commodity, and also facilitate environmental safety.

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References
[1] Gullo M and Giudici P 2008 Acetic acid bacteria in traditional Balsamic vinegar: phenotypic traits relevant for starter cultures selection. Int. J. Food Microbiol 125 46-53.
[2] Turker I 1963 Sirke Teknolojisi ve Teknikte Laktik Asit Fermantasyonları. Schoolbook of Faculty of Agriculture Ankara Univ 181.
[3] Tan S C 2005 Vinegar fermentation [Master of Science thesis]. Louisiana State University Department of Food Science Baton Rouge 101.
[4] Fushimi T and Sato Y 2005 Effect of acetic acid feeding on the circadian changes in glycogen and metabolites of glucose and lipid in liver and skeletal muscle of rats. Br. J. Nutr 94 714-9.
[5] Kondo S, Tayama K, Tsukamoto Y, Ikeda K and Yamori Y 2001 Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. Biosci. Biotechnol. Biochem 65 2690-4.
[6] Xu Q, Tao W and Ao Z 2007 Antioxidant activity of vinegar melanoids. Food Chem 102 841-
9.
[7] Adams M R 1985 Microbiology of fermented foods. *New York Institute of Food Technologists* 1-45.

[8] Korkerd S, Wanlapa S, Puttanek C, Uttapap D and Rungsardthong V 2016 Expansion and functional properties of extruded snacks enriched with nutrition sources from food processing by-products. *J. Food Sci. Technol.* 53 (1) 561–570.

[9] Kasikorn Research Center 2011 Thai rice innovations. from http://www.kasikornresearch.com.

[10] Moonsarn Y and Moonsarn P 2011 Improvement of noodle waste by yeast for use as animal feed. *J. Sci.* 2 1905-3193.

[11] Tsuyoshi N, Fudou R, Yamanaka S, Kozaki M, Tamang N, Thapa S and Tamang J P 2005 Identification of yeast strains isolated from marcha in Sikkim: a microbial starter for amylolytic fermentation. *Int. J. Food Microbiol.* 99 135-146.

[12] Limtong S, Sintara S, Suwanarit P and Lotong N 2005 Species diversity of molds in Thai traditional fermentation starters (Loog-Pang). *KU J. Soc Sci.* 39 511-518.

[13] Limtong S, Sintara S, Suwanarit P and Lotong N 2002 Yeast diversity in Thai traditional alcoholic starter. *KU J. Soc Sci.* 36 149-158.

[14] Chou C C and Rwan J H 1995 Mycelial propagation and enzyme production in koji prepared with *Aspergillus oryzae* on various rice extrudates and steamed rice. *J. Biosci. Bioeng.* 79 509-512.

[15] Miller G L 1959 Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.* 31 426-429.

[16] AOAC International 2000 Official Method of Analysis, Association of Official Agricultural Chemist. 17th Ed. AOAC International. Gaithersburg: MD.

[17] Woiciechowskı A L, Nitsche S, Pandey A and Saccol C R 2002 Acid and enzymatic hydrolysis to recover reducing sugars from cassava bagasse: an economic study. *Braz. arch. biol. technol.* 45 393-400.

[18] Amnuaycheewa P, Rodiahwati W, Sanvarinda P, Cheenkachorn K, Tawai A and Sriariyanun M 2017 Effect of organic acid pretreatment on napier grass (*Pennisetum purpureum*) straw biomass conversion. *KMUTNB Int. J. Appl Sci. Technol.* 10 107-117.

[19] Dung N T P, Rombouts F M and Nout M J R 2006 Functionality of selected strains of moulds to recover reducing sugars from cassava bagasse: an economic study. *Braz. arch. biol. technol.* 45 393-400.

[20] Hernández-Ort e P, Ibarz M J, Cacho J and Ferreira V 2006 Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. *Food Chem.* 98 300-10.

[21] Negi S and Benerjee R 2006 Optimization of amylase and protease production from *Aspergillus awamori* in single bioreactor through EVOP factorial design technique. *Food Sci. Biotechnol.* 44 257-61.

[22] Taillandier P, Portugal F R, Fuster A and Strehaiano P 2007 Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content. *Food Microbiol.* 24 95-100.

[23] Byarugaba-Bazirake G, Byarugaba W, Tumusime M and Kimono D A 2014 The technology of producing banana wine vinegar from starch of banana peels. *Afr. J. Food Sci. Technol.* 5 1-5.

[24] Oyotor O O, Denubi E, Ogundipe O O, Bankole B O and Olalekan Adeyeye S A 2017 Production and quality evaluation of vinegar from mango. *Cogent Food Agric.* 3 1278193.

[25] De Ory I, Romero L E and Cantero D 2002 Optimum starting-up protocol of a pilot plant scale acetic acid vinegar production. *J. Food Eng.* 52 31-7.

[26] Drysdale G S and Fleet G H 1988 Acetic acid bacteria in winemaking: a review. *Am. J. Enol. Vitic.* 39 (2) 143-154.

[27] González A, Hierro N, Poblet M, Mas A and Guillamon J M 2005 Application of molecular methods to demonstrate species and strain evolution of acetic acid bacteria population during wine production. *Int. J. Food Microbiol.* 102 295-304.