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A study on the volatile compounds in Japanese alcoholic beverage (Awamori) produced from Thai rice varieties

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Abstract

Awamori is a famous alcoholic beverage brewed in the Southern part of Japan. Thai rice is well known and there are many varieties of rice including white rice and pigmented rice. This research aimed to study the properties and volatile compounds of Awamori produced from five Thai rice varieties including KDML105, Chainat, RD6, brown KDML105, and riceberry rice. Rice koji was prepared by inoculating the steamed rice with tane-koji from *Aspergillus awamori* at 0.2% by weight. Then, water and *Saccharomyces cerevisiae* were added and alcoholic fermentations were performed at around 2 weeks. The production of alcohol and pH changes during the liquid fermentation were monitored, and volatile compounds of the Awamori were determined by gas chromatography-mass spectrophotometry (GC-MS). The highest soluble solid and alcohol content were observed in the fermentation of KDML 105. The alcohol content after 2 weeks of fermentation ranged from 14.5-18 %, with the pH around 4.02-4.5. The same volatile compounds, ethyl alcohol, isobutyl alcohol, isoamyl alcohol, phenylethyl alcohol, isoamyl acetate, ethyl laurate, ethyl linoleate, with the flavor descriptions as alcohol, wine-like, mushroom-like, floral, rose, fruity, banana, sweet, ice-cream, mild fatty, oily, and honey-like odors were detected in all samples from Thai rice while KDML105 presented greater diversity of volatile compounds than the other Thai rice.

*Keywords***:** *Awamori; Japanese sake; rice koji; Thai rice; volatile compound; pigmented rice*

1. Introduction

Sake is the most popular traditional Japanese alcoholic beverage, brewed by simultaneous saccharification and alcohol fermentation (Li et al., 2020). Japanese sake was the second most popular beverage in Shiga, Kyoto, and Yamaguchi prefectures (Ohno, 1995) while Awamori is the favorite sake in Okinawa. Awamori is the Japanese fermented and distilled sake made from rice, popularly brewed in the Kyushu area in South Japan (Li et al., 2020). Formerly, the rice type used for awamori fermentation is indica rice which had been brought from Thailand in the fifteenth century. Currently, Awamori is one of the staple alcoholic beverages in Japanese culture, brewed from Japanese rice, which indicates around 60-86 proof (30-46% alcohol). More than 40 local distilleries are producing different kinds of Awamori in Okinawa (Tsuta et al., 2017) using different fermentation and distillation techniques as well as aging periods to produce specific quality and flavor of their products (Tamamura, & Higa, 2009).

Rice (*Oryza sativa* L.) is widely cultivated and consumed as a staple food by over half of the world's population, especially in Asian countries (Tangsrianugul, Wongsagonsup, & Suphantharika 2019). The main component in rice flour is a carbohydrate that is used as energy for human growth. Rice is also a good source of protein, lipids, fiber, and some vitamins (Ito, & Lacerda, 2019). Generally, there are two types of rice based on their texture, as glutinous rice and non-glutinous rice, which are mainly related to their amylopectin content. In Thailand, the main rice varieties are white rice generally consumed as milled white rice, and brown or un-milled rice. Many species of rice contain pigments based on their pericarp color such as black, purple, and red rice. Several works have reported the antioxidant activity of phenolic compounds and anthocyanin in pigmented rice, which is beneficial to human health (Tangsrianugul et al., 2019). Brown rice has higher nutritional values than its white counterparts and is healthier. Riceberry rice presents high concentrations of antioxidants such as vitamin E, polyphenols, and phytosterols, that could help against diabetes. White rice flour can be used for many processing products including rice noodles and rice pasta (Charutigon et al., 2008; Sereewat et al., 2015, Rungsardthong et al., 2021). Thai rice malt was investigated for the production of non-alcoholic beer (Puangwerakul et al., 2014). Glutinous rice is also used in fermented food products including the traditional Thai fermented alcoholic beverage "Sato" and fermented glutinous rice called "Khao Mak" (Wongsa, Rungsardthong, & Yasutomo, 2018).

Awamori fermentation can be achieved by inoculating steamed rice with the black fungi, *Aspergillus awamori* to prepare rice koji. During the incubation, *A. awamori* produces various enzymes such as α-amylase, glucoamylase, and protease that are required for the solid-state saccharification process. The degree of saccharification in koji during the solid state is about 70% of starch (Zhao et al., 2021). Water and *S. cerevisiae* are then added to the rice koji so that yeast can produce alcohol from soluble disaccharides and monosaccharides in the liquidstate fermentation which takes around 10 days before the distillation process (Zhao et al., 2021). Several aroma compounds contributing to the quality of Japanese sake including Awamori made from Japanese rice were analyzed and reported (Osafune et al., 2020, Li et al., 2020, Zhao et al., 2021, Sasaki et al., 2023).

There are many compounds, including sugars, amino acids, gamma aminobutyric acid (GABA), organic acids, and volatile compounds in Japanese sake. Aromatic compounds in the alcoholic beverage can be identified by gas chromatography-mass spectrometry (GC-MS) with various techniques such as liquid-liquid extraction (Blanch, Reglero, & Herraiz, 1996), solid-phase extraction (López et al., 2002), and solid-phase micro-extraction (SPME) (Lee et al., 2001). Many of the low molecular compounds in sake are odors and taste-active compounds. However, to the best of our knowledge, there are no reports on the fermentation and aroma profile of Awamori prepared from Thai rice (as well as pigmented rice) before. The type and properties of the ingredients including rice, water, and microbial strains significantly affect the properties, quality, as well as aroma profile of the obtained Awamori.

2. Objective

The objective of this study was to investigate the use of five varieties of Thai rice including KDML105, Chainat, RD6, Brown KDML105, and riceberry rice for the production of Awamori. Some properties such as alcohol percentage, and pH changes during the fermentation were monitored. The fermentation liquid was distilled and analyzed for its aroma profiles using a gas chromatography-mass spectrometry (GC-MS). The information obtained will be beneficial for the alternative use of Thai rice for the fermentation of Awamori.

3. Materials and methods

3.1 Microbial strains

Saccharomyces cerevisiae was cultured on the yeast malt (YM) agar slant for 1 week before subculturing one loop of this seed-culture to another YM agar slant and incubated at 28 ºC for 24 h*.* For *Aspergillus awamori*, the seed-culture was prepared by inoculating one loop of the *A. awamori* mycelium to potato dextrose agar (PDA) slant and incubated at 28 ºC for 1 week. YM and PDA were the products from Difco Laboratories (Detroit, MI, USA). Then, the agar slant was covered with the green fungus spores of *A. awamori*. To prepare the tane-koji, firstly 30 g of rice grains with 5 mL of distilled water was placed in a 200 mL Erlenmeyer flask and sterilized in an

autoclave (LSX-500, TOMY, Japan) at 121ºC for 15 min. One loop of the *A. awamori* spores from the seed-culture was inoculated to the sterilized rice grains and incubated at 28 ºC for 2 weeks until the rice grains were covered with the green spores.

3.2 Preparation of Awamori from Japanese rice and two varieties of Thai rice

One Japanese rice and two Thai rice varieties (Figure 1) were used for the Awamori fermentation at the Department of Bioresource Technology, Okinawa National College of Technology, Okinawa (Japan). Hitomebore, the Japanese rice was purchased from a supermarket in Okinawa, Japan.The white glutinous rice, RD6, was obtained from the Bureau of Rice Research and Development, Phitsanulok, while the purple glutinous rice, Kam Doi, was purchased from a local market in Lampang (Thailand).

Figure 2 presents a schematic flow for the production of Awamori using Hitomebore, RD6, and Kam Doi at the Okinawa National College of Technology, Japan. Koji was prepared from each rice variety. In brief, polished rice was washed until the water was clean and soaked in water for 1 h. Excess water was removed from rice before the steaming at 121ºC for 15 min using an autoclave (LSX-500, TOMY, Japan). After cooling to around room temperature, the steamed rice was inoculated with Tane-koji at 0.2% by weight. The inoculated rice was incubated at 30°C for around 48 h in the incubator (IW-450 (AS ONE, Japan) until the white mycelium covered the surfaces of the rice. Then, approximately 400 g of the prepared koji was transferred into a 1 L flask containing 900 mL of water and 5 mL of the yeast seed-culture, *S. cerevisiae*. The fermentation was performed at room temperature (25-30ºC) for 21 days. The fermentation liquid was measured for its pH with a pH meter (Seven Multi, METTLER TOLEDO, Japan) and alcohol percentage by the GC-MS, QP2010 Ultra (Shimadzu, Japan) with the column, Stabilwax (60 m x 0.25 mm x 0.50 um). The column temperature started from 60℃ and increased to 200℃ at the rate of 20℃/min. Ionization was performed at 200℃ and the carrier gas was He (23 cm/sec). The injection volume was 3 µL. The alcohol percentages are presented as % v/v.

3.3 Preparation of Awamori from five varieties of Thai rice

Four milled non-pigmented rice varieties; KDML-105, Chainat, RD6, brown KDML105, and one pigmented rice, riceberry rice purchased from a supermarket in Bangkok (Thailand) were used for the Awamori fermentation at the Department of Agro-Industrial, Food and Environmental Technology, King Mongkut's University of Technology North Bangkok (KMUTNB), Bangkok (Thailand). The chemical compositions of five rice varieties used in the fermentation were determined following AOAC method (2000) for their protein, lipid, while carbohydrate was determined by difference method. All determinations were carried out in triplicate. The chemicals used were all analytical grade.

The preparation of rice koji and Awamori fermentation were performed according to the process described in 3.2 and Figure 2. However, after soaking, each rice was steamed at 100ºC for 30 min instead of the high-pressure steaming to avoid excessive adhesiveness of the rice after the sterilization. The fermentation of the mash with *S. cerevisiae* was carried out at room temperature (27- 31ºC) for 15 days. During the fermentation, alcohol percentage was measured using a winometer (LX-013-A, China), pH-meter (Model 510, Cyberscan, Netherland), and soluble solids by a hand refractometer (HR-130, Optika, Italy).

Figure 1 Three rice varieties (raw) used for the production of Japanese alcoholic beverage, Awamori

Figure 2 Schematic illustration of the production of Awamori from each variety of rice

3.4 Volatile profiles of distilled Awamori prepared from five varieties of Thai rice

Distillation was performed at $92 \pm 2^{\circ}$ C. This setup was repeated 4 times to increase throughput. After distillation, the resulting liquid was combined into a flask that was then sealed. Distillation of each fermentation was carried out as described above. The combined alcoholic beverage (around 40% alcohol), Awamori, from the fermentation of five Thai rice varieties was diluted to be about 20% alcohol and analyzed for their volatile compounds using a GC-MS followed Yin et al. (2020) and Osafune et al. (2021). The analysis was performed using A 8890 GC-system coupled with 59778 GC/MSD 59778 (Agilent Technologies Inc., Santa Clara, CA, USA) with a capillary column HP-5MS UI (60 m \times 0.25 mm i.d. \times 0.25 μm film thickness) and a split ratio of 1:1. Twenty mL of the distilled Awamori was filled in a 50 mL vial and pre-incubated at 50°C for 5 min. The headspace extraction temperature and time were 50 °C for 30 min, respectively, before the injection into the GC column. The system was equipped with the headspace solid-phase microextraction (HS-SPME), and a Teflon-lined septa, 50/30 μ m divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco Inc., Bellefonte, PA). The analysis program was: oven temperature set initially at 45 °C for 5 min, then increased from 45 to 250 °C at a rate of 5 °C/min. The Electronimpact mass spectra were generated at 70 eV, with m/z scan range from 35 to 300. The temperature of the MS source was controlled at 250 °C. Volatile compounds in each sample were identified with the reference of NIST 14 mass spectra libraries (Yang et al*.*, 2015) installed in the GC-MS equipment, while their odor descriptions were presented followed published reports (Xiang et al., 2020; Yin et al., 2020; Zhao et al., 2021)

4. Results and discussions

4.1 Production of Awamori from Japanese rice and two Thai rice varieties

The total amounts of alcohol produced and pH changes in the fermentation by three rice varieties during 21 days were very similar as shown in Figure 3. The alcohol content increased sharply to 7.28-7.66% after 1 week and remained at 8.9- 9.4% at Day 21. The pH in both fermentations from Hitomebore, and Thai rice, RD6, and Kam Doi was not much different, ranged 2.91-4.02, and 2.85- 3.81, respectively, from Day 0-Day 21 while the pH in the fermentation from Kam Doi started from 3.05 and increased to 4.95 at Day 21. The low pH in the fermentation liquid at Day 0 was due to the acid metabolites from the fungus growth in the koji. The solid-state saccharification contributes to the production of sugars (Xiaowei, & Han, 2016) which will be converted to alcohol by *S. cerevisiae* during the liquid fermentation. Many metabolites were produced while some acid metabolites were also

formed in which the major acid in Awamori was citric acid (Zhao et al., 2021).

Figure 4 presents the appearance of the liquid fermentation of Awamori (before the alcohol distillation) prepared from Kam Doi, Hitomebore, and RD6 on Day 21, which contained alcohol content at 9.4%, 8.9%, and 9.2%, respectively. The fermentation liquid from the pigmented rice, Kam Doi, exhibited reddish brown color. The color was derived from the pigments on the rice pericarp which usually contains phenolic compounds and anthocyanins that indicate health benefits (Tangsrianugul et al., 2019). However, the distilled alcohol beverages from all rice varieties, both Japanese and Thai rice varieties were transparent and showed good aromas. The results showed that Thai rice is also good for the production of Awamori. As described above Thai indica rice was originally used for awamori fermentation in the former time.

4.2 Chemical compositions of five Thai rice varieties

Chemical analysis of KDML105, Chainat, RD6, brown KDML105, and riceberry rice exhibited their protein at 7.36%, 7.27%, 6.82%, 8.13%, and 8.99% while their carbohydrate content determined by difference method was 81.51%, 78.19%, 84.05%, 77.15%, and 77.35%, respectively. The lipid content in each rice variety was 1.59%, 1.07%, 0.72%, 2.59%, and 2.93%, respectively. Different compositions in the rice which is the main raw material for the fermentation would influence the quality and aroma profiles of each distilled Awamori obtained.

4.3 Production of Awamori from five Thai rice varieties

The appearance of Awamori fermentation from five Thai rice varieties before the alcohol distillation are presented in Figure 5. Figure 6 shows the increasing soluble solids during the liquid fermentation for 15 days. Although sugars were converted to alcohol by the yeast, the total soluble solids increased with time. The sugars obtained from the starch hydrolysis dissolved into the fermentation liquid and increased the total soluble solids rapidly. Soluble solids were increased rapidly during the first three days for all fermentations. It seemed that saccharification occurred very well with KDML105. The highest soluble solid concentration, which increased up to

12% on day 15, was observed when KDML105 was used while only 8-9% was obtained for another four rice varieties.

Some differences in the pH of the fermentation at Day 0 were observed. This could be due to the different chemical composition, degree of starch and protein hydrolysis of each rice variety during the koji preparation. The pH of the fermentation was lowered and kept around 4.0-4.3 during the fermentation for 15 days. The alcohol content in the fermentation after adding water and *S. cerevisiae* to each rice koji increased rapidly for the first week of incubation. The final alcohol concentration for the fermentation of four nonpigmented rice was about 15-16% at Day 15 while that of riceberry was only about 14.2%. The alcohol production level was influenced by various factors such as pH, medium composition, and the sugar content of the mash (Fleet, 1993; Caridi, Crucitti, & Ramondino, 1999). Chuenchomrat, Assavanig, & Lertsiri (2008) prepared a traditional Thai rice wine (Ou), started from the solid-state saccharification using a starter which was composed of a mixed culture of mold and yeast. After the saccharification, water was added to let the yeasts in the starter convert sugar to alcohol. The ethanol content in the fermentation liquid was in the range of 1.21-10.46 % (w/v). The development of Sato, another Thai-rice based alcoholic beverage (Luangkhlayphoa et al., 2014), using NP1 starter and the defined starter culture mixture presented that the alcohol concentration in the liquid fermentation increased sharply during day 3-5 from 5.7-6% (v/v) up to 10-11% (v/v) and reached the maximum at about 13-15% (v/v) on day 13 for both starters.

Different rice varieties compose of different structures and chemical compositions, especially starch content and protein as presented above. Riceberry rice indicates a higher gelatinization temperature and a lower swelling power than KDML105 [\(Thiranusornkij](https://pubmed.ncbi.nlm.nih.gov/?term=Thiranusornkij+L&cauthor_id=30722865) et al., 2019). The comparative studies on the physicochemical properties of riceberry rice starch and KDML105 starch showed that riceberry rice starch contained higher contents of resistant starch (RS) and lower contents of slowly digestible starch (SDS). The starch properties mainly affect the saccharification in the solid state and soluble solid as well as alcohol content in the liquid fermentation.

4.4 Volatile profiles of Awamori produced from five Thai rice varieties

The volatile compounds in distilled Awamori from Thai rice varieties identified by GC-MS are presented in Table 1 and compared with the volatile compounds of Japanese Awamori. There are several alcohol, aldehyde, ester, and acid compounds identified from Awamori from Japanese rice as listed in Table 1 (Yin et al., 2020; Osafune et al., 2021). Aroma compounds in Chinese distilled liquor and Japanese distilled liquors, Awamori, were characterized and compared in previous study (Osafune et al., 2020; Xiang et al., 2020; Yin et al., 2020; Zhao et al., 2021). The flavor of Awamori is mainly expressed for their flavor description as mushroom-like, kojilike, fruity, and sweet flavors (Miyamoto, 2018). Osafune et al., (2020) reported that Awamori presented a fruity, sweet, characteristic mushroomlike flavor, strong sour, koji-like, oily, and cereallike aromas. Sixteen key compounds selected based on the concentration found in Awamori competition such as isovaleraldehyde, hexanal, ethyl butyrate, citronellol, and linalool.

The main volatile compounds in Awamori from each Thai rice and Japanese rice present the main compounds as alcohol, aldehyde, ester, and acid compounds. Ethyl alcohol, isobutyl alcohol, isoamyl alcohol, phenylethyl alcohol, isoamyl acetate, ethyl laurate, ethyl linoleate, which can be described for its flavor as alcohol, wine-like, mushroom-like, floral, rose, fruity, banana, sweet, ice-cream, mild fatty, oily, and honey-like odors were detected in all samples from Thai rice and Japanese rice. However, each Awamori sample also contained different volatile compounds which led each product to have fruity, sweet flavor and their specific flavors. Though the same fungus strain, *A. awamori* and yeast, *S. cerevisiae*, were used for the koji preparation and liquid fermentation, different volatile compounds were identified in the Awamori from different rice variety. The chemical composition of each rice variety can lead to different ingredient for the growth and conversion into different compounds and flavor profiles. The esterification of ethanol and the organic acids, isobutyric acid, isovaleric acid and lactic acid, lead to the formation of ester, ethyl isobutyrate, ethyl isovalerate and ethyl lactate, respectively, in the fermentation liquid (Rahayu et al., 2017).

Figure 4 Appearance of Awamori fermentation from Thai purple glutinous rice-Kan Doi (left), Japanese rice-Hotmebore (middle), and Thai glutinous white rice-RD6 before the alcohol distillation

Figure 5 Appearance of Awamori fermentation from five Thai rice varieties before the alcohol distillation; KDML105(A), Chainat (B), RD6 (C), brown KDML105 (D), and riceberry rice (E)

Figure 6 Changes in total soluble solid (°Brix), pH of and alcohol content (%) in the Awamori fermentation from five Thai rice varities

Table 1 Volatile compounds in distilled Awamori from five Thai rice varieties analysed by GC-MS.

* Yin et al., (2020) and Osafune et al., (2021)

** Xiang et al., (2020), Yin et al., (2020) and Zhao et al., (2021)

5. Conclusion

This study focused on the use of several Thai rice varieties both white rice and pigmented rice to produce the Japanese distilled alcohol beverage, Awamori, which is popularly consumed in the Southern part of Japan. The alcohol content and pH changes during the liquid fermentation were monitored. The results showed that the physical properties, alcohol content and flavor profiles of the Awamori from five Thai rice were acceptable. Several volatile compounds of the Awamori from Thai rice varieties were identical with the Japanese Awamori, revealing fruity, mushroom-like, and sweet flavors, with some other typical specific flavor profiles depending on the use of different Thai rice varieties. The study confirms the potential of using Thai rice for the production of the Japanese Awamori.

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454