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Philippine rice wine (*Tapuy*) made from *Ballatinao* black rice and traditional starter culture (*Bubod*) showed high alcohol content, total phenolic content, and antioxidant activity

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Abstract

This study described and evaluated the acidity, alcohol content, total phenolic content, antioxidant activity, and sensory attributes of rice wine (*Tapuy*) made from black, red, brown and white rice during a 7-day fermentation. Acidity of *Tapuy* made from the different pigmented rice had similar pH profiles characterized by a steep decrease at day 1 from 6.38 ± 0.4 to 3.38 ± 0.2, and remained relatively unchanged until the end of fermentation. *Tapuy* made from black rice (*Ballatinao*) contained 16.07% alcohol, second to white rice (*Bongkitan*) that contained 18.58% alcohol, although they were not significantly different from each other (p>0.05). At the end of fermentation, the total phenolic content and antioxidant activity of *Tapuy* made from black rice was significantly higher (p<0.05 and p<0.01, respectively) than *Tapuy* made from the other pigmented rice. Total phenolic content of *Tapuy* made from black rice was at 9.73 mg GAE/mL and antioxidant activity leveled-off at 70.63% DPPH radical scavenging activity; whereas *Tapuy* made from the other pigmented rice were declining. Thus, *Ballatinao* black rice is a suitable rice variety for making *Tapuy* and has the potential to be a health-promoting functional food.

Keywords: antioxidant activity; *Ballatinao* black rice; fermentation; rice wine.

Practical Application: Use of *Ballatinao* black rice is a healthier alternative than glutinous white rice to make *Tapuy*.

1 Introduction

Fermentation causes many biochemical changes in food products that can alter the ratio of nutritive and anti-nutritive components resulting to altered properties of the food material, such as bioactivity and digestibility (Zhang et al., 2012). Increasing consumer awareness with diet-disease relationships have attracted scientific interest in fermentation and how it can increase the health-promoting benefits of food products (Xiang et al., 2019). Fermentation can improve the antioxidant activity of food products by increasing the release of bioactive phenolic compounds and flavonoids in plant-based products (Hur et al., 2014; Lee et al., 2008). Fermentation can also produce probiotic-rich food products that can have positive effects on memory enhancement by improving gut health properties (Gobbetti et al., 2010; Selhub et al., 2014). The health-promoting benefits, particularly antioxidant activity, of fermented food products can be attributed to the carbohydrate-metabolizing enzymes produced during fermentation that promotes the bioconversion of phenolic glycosides into their free form, i.e. aglycones, which have high antioxidant activity (Vattem & Shetty, 2003).

The Philippines has many traditional fermented food products including fermented rice, fermented fish, fermented mustard leaves, fermented green mango, coconut wine, palm vinegar and many others (Sanchez, 1999). *Tapuy* is a traditional fermented food product popular in the Northern part of the Luzon island specifically Benguet, Ifugao, and the Mt. Provinces (Sakai & Caldo, 1985). It is an alcoholic sweet-acidic beverage that is produced by the simultaneous saccharification and fermentation of glutinous white rice (Sanchez, 2008). *Tapuy* is produced using the traditional starter culture, *Bubod*, which are dried discs of ground glutinous rice, wild grass roots (*Bidens pilosa*), ginger and old *Bubod* (Sanchez, 1999). This local rice wine is part of the culture and tradition of the Cordillera people who consume it during festivities and traditional ceremonial occasions (Bandonill et al., 2009; Sanchez, 1999). The traditional methods of making *Tapuy* vary in the different Northern Luzon provinces which resulted in the various quality of *Tapuy* from one maker to the other and from one locality to another (Tanimura et al., 1977). All these methods of making *Tapuy* have been developed on a trial and error basis, passing down this *Tapuy* making process from one generation to another (Sanchez, 1999). Several efforts have been made in producing consistent quality for *Tapuy* such as development of protocols for the standardization of *Tapuy* production for commercial purposes headed by the Philippine Rice Research Institute (PhilRice) (Ablaza et al., 2008; Bandonill et al., 2009).

Rice (*Oryza sativa* L.) is the most important cereal crop produced in Asia that is consumed as a staple food by nearly half of the world's population (Huang & Lai, 2016; Priya et al., 2019). Rice cultivars exists in different colors such as white, brown, red, purple and black (Kushwaha, 2016). White rice is the rice cultivar most consumed by humans (85%) while the rest are the colored or pigmented rice (Ling et al., 2001). Pigmented rice, however, are nutritionally superior and have richer taste compared to common white rice (Kushwaha, 2016; Su et al., 1998). Pigmented rice contains

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anthocyanins and proanthocyanidins which have antioxidant (Rattanachitthawat et al., 2010), anti-inflammatory (Wang et al., 2007), cardioprotective and antiatherogenic activities (Ling et al., 2001). Pigmented rice was historically cultivated in Southeast Asian countries, however, China and India, including Thailand, are now the major producers of pigmented rice (Kong et al., 2008; Prasad et al., 2019). In the Philippines, *Ballatinao* is a glutinous black rice variety found in the northern regions of the country. It is considered as an heirloom rice by the indigenous people of the Cordillera and is served during ceremonial festivities and traditional practices (PhilRice, 2017). Compared to the local red rice and non-pigmented rice, *Ballatinao* black rice had the highest anthocyanin content, total phenolics, crude protein and crude fat (Romero et al., 2012). Black rice varieties generally have high anthocyanin content (Hou et al., 2013), and several countries in Asia are exploring this feature to produce rice wine with high antioxidant activities, including India, Japan, Korea, China, Thailand, and Cambodia (Chay et al., 2020; Jiang et al., 2020b; Kim et al., 2012; Moirangthem et al., 2020; Singkong, 2015; Takeshita et al., 2015). In the Philippines, *Ballatinao* black rice is already used in *Tapuy* making, but to our knowledge, no studies have been done describing the use of this black rice variety in *Tapuy* production. Utilization of the *Ballatinao* black rice in *Tapuy* production can help promote the local heirloom rice industry and preserve the cultural practices of the Cordillera people surrounding this heirloom rice. This study characterized the acidity, alcohol content, total phenolic content, antioxidant activity, and sensory attributes of *Tapuy* made from *Ballatinao* black rice, *Kintoman* red rice, *Tinawon* brown rice, and *Bongkitan* white rice during a 7-day fermentation.

2 Materials and methods

2.1 Starter culture

Traditional starter culture, or *Bubod*, was purchased at a local market in Baguio City, Philippines.

2.2 Rice grains

Two glutinous rice varieties – black rice (*Ballatinao*) and white rice (*Bongkitan*), and two non-glutinous rice varieties – brown rice (*Tinawon*) and red rice (*Kintoman*), were purchased at a local market in Baguio City, Philippines (Figure 1).

2.3 Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Ascorbic Acid was purchased from Chemline Scientific Corporation (Quezon City, Philippines). Potassium Dichromate ($K_2Cr_2O_7$) and Sulfuric acid (H_2SO_4) were provided by the Department of Biochemistry and Molecular Biology Laboratory, University of the Philippines Manila.

2.4 Fermentation procedure

Tapuy was prepared as follows: 5 g of uncooked rice and 10 mL of deionized water were placed into a 50 mL Erlenmeyer flask and autoclaved at 121 °C for 15 minutes. After cooling, 2 g of ground *Bubod* and 8 mL of deionized water were added into the Erlenmeyer flask and mixed with the cooked rice. The Erlenmeyer flasks were sealed using cork and placed in an incubator set at 25 °C. Rice wine samples were then collected every day, from day 0 until day 7 of fermentation. This was done by filtering the mash made from the rice grains collected. The collected rice wine, or *Tapuy*, was then analyzed.

2.5 Determination of acidity

The acidity of the *Tapuy* was determined using an Orion pH meter MDI 420A (Vernon Hills, IL, USA).

2.6 Determination of alcohol content

Alcohol content of the *Tapuy* was determined by the Ethanol-Dichromate assay based on the method of Sumbhate et al. (2012). 0.25 M $K_2Cr_2O_7$ and 6 M H_2SO_4 were prepared and 50 µL each of the solution was mixed together and added to 100 µL of *Tapuy*. The reaction was allowed to occur for five minutes and then read at 570 nm against a standard curve generated from different concentrations of ethanol.

2.7 Determination of total phenolic content

Total phenolic content of the *Tapuy* was determined by Folin-Ciocalteu method with modifications. 15.4 µL of wine samples and gallic acid standard (at different concentrations) were mixed with 61.5 µL of Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and were neutralized with 123 µL of 7.5% sodium carbonate. The mixtures were allowed to stand at

Figure 1. Pigmented rice varieties used in the study. (A) White rice (*Bongkitan*); (B) Brown rice (*Tinawon*); (C) Red rice (*Kintoman*); (D) Black rice (*Ballatinao*). These heirloom pigmented rice varieties were cultivated at high altitude areas in the Cordillera region and are sold at local markets in the Cordillera provinces.

room temperature for 30 minutes. The absorbance was measured at 765 nm. The results were expressed as gallic acid equivalents (mg GAE/mL).

2.8 Determination of antioxidant activity

Antioxidant activity of the *Tapuy* was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay based on the method of Prieto (2012). 0.2 mM DPPH solution in 95% ethanol was prepared and 100 µL of this solution was added to 100 µL of *Tapuy*. The reaction was allowed to occur for 30 minutes in the dark and then read at 517 nm. DPPH radical scavenging activity was obtained using the following Formula 1:

$$\%\text{DPPH Radical Scavenging Activity} = 100\% \times \frac{Abs_{DPPH+Water} - Abs_{DPPH+Sample}}{Abs_{DPPH+Water}} \quad (1)$$

where $Abs_{DPPH+Water}$ is the absorbance of the control reaction and $Abs_{DPPH+Sample}$ is the absorbance of the *Tapuy* sample.

2.9 Sensory evaluation

Sensory evaluation of the *Tapuy* made from different pigmented rice was based on the method of Chay et al. (2017). 15 mL of wine samples were dispensed in a clean shot glass coded with 3-digit random numbers and placed on serving plates. 20 panelists recruited among students, faculty and staff members of the University of the Philippines, Manila (Manila, Philippines), evaluated the sensory attributes of the *Tapuy*. The panelists were asked for preferences to color, clarity, aroma, sweetness, sourness, and bitterness using a score sheet with a scale of 1 to 9, where: Color: 1=Extremely light color, 9=Extremely dark color; Clarity: 1=Extremely clear, 9=Extremely cloudy; Aroma; 1=Extremely weak, 9=Extremely strong; Sweetness: 1=Extremely not sweet, 9=Extremely sweet; Sourness: 1=Extremely not sour, 9=Extremely sour; Bitterness: 1=Extremely not bitter, 9=Extremely bitter.

2.10 Statistical analyses

All statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY, USA). All experiments were done in triplicates. Analysis of variance (ANOVA) was performed using the general linear model procedure to determine significant differences among the samples. Means were compared by using Tukey's honest significant difference (HSD) test. Significance was defined at the 5% level.

3 Results and discussion

3.1 Tapuy made from different pigmented rice have similar acidity profiles

The pH of *Tapuy* made from different pigmented rice are shown in Figure 2. The different rice wines exhibited a pH range of 2.94 to 3.60 during the 7-day fermentation, with a final pH range of 3.16 to 3.40. These pH values were not significantly different from each other ($p>0.05$), and fall within the pH range (3.01-3.74) of indigenously-produced commercial *Tapuy* from Benguet, Philippines (Hipol & Alma-in, 2018). Similar pH values were also exhibited by rice wines made from pigmented rice from other Asian countries (Baek et al., 2013; Takeshita et al., 2015; Teramoto et al., 2011). A steep decrease in pH from day 0 to day 1 of fermentation was noted for all the different *Tapuy* (Figure 2). This steep decrease in pH was not unusual since it has also been observed during the fermentation of *Makgeolli*, a Korean traditional rice wine (Kim et al., 2013). This decrease in pH of the *Makgeolli* was associated with the production of organic acids by microorganisms (e.g. lactic acid bacteria) during the first day of fermentation (Jin et al., 2008; Lee et al., 2009). Lactic acid bacteria were also found in *Tapuy* which were made using the traditional starter culture (*Bubod*) (Sanchez, 1999), the same starter culture used in this study. The weakly acidic pH of *Tapuy* may also be attributed to the production of carbonic acids from the reaction of alcohol and CO_2 with water during fermentation (Singkong, 2015). The acidity, coupled with the presence of volatile compounds, affect the flavor quality and shelf life of rice wines (Lee et al., 2009).

3.2 Tapuy made from black and white rice had the highest alcohol content

The alcohol content of *Tapuy* made from different pigmented rice are shown in Figure 3. There was an increase in the alcohol content of the different rice wines during the 7-day fermentation. The final alcohol content of the rice wines after fermentation was 9.13 to 18.58% (Figure 3). These values fall within the range of alcohol contents (8.19-19.83%) of commercial *Tapuy* from Benguet, Philippines (Hipol & Alma-in, 2018). Similar alcohol contents (14-18%) were also shown in *Makgeolli* (Jin et al., 2008) and the traditional Guizhou black glutinous rice wine from China (Jiang et al., 2020b). The increase in alcohol content during fermentation was expected as it is brought about by the concerted action of molds and yeast in hydrolyzing rice starch into glucose and converting glucose into ethanol, respectively (Jiang et al., 2020a; Kim et al., 2013). *Bubod* from the Cordillera region contains molds and yeasts (e.g. *Aspergillus oryzae,*

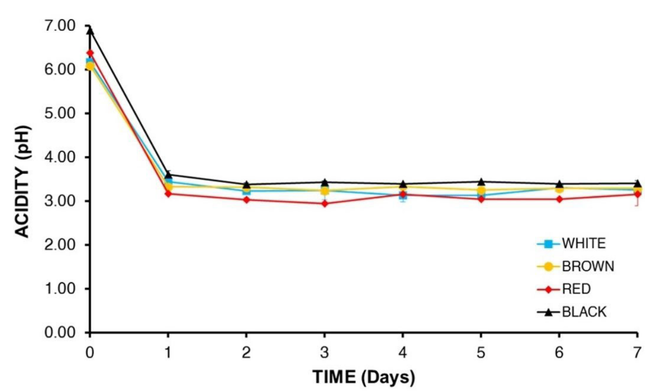

Figure 2. Acidity (pH) of *Tapuy* made from different pigmented rice during the 7-day fermentation. Data points are the mean of 3 replicates. Error bars are the standard deviation for each data point. *Tapuy* made from different pigmented rice exhibited a pH range of 2.94 to 3.60 during the 7-day fermentation. The pH profiles of all *Tapuy* showed a steep decrease in pH from day 0 to day 1 and remained relatively unchanged throughout fermentation. The final pH of the different *Tapuy* range from 3.16 to 3.40 which were not significantly different from each other ($p>0.05$). *p-value <0.05.

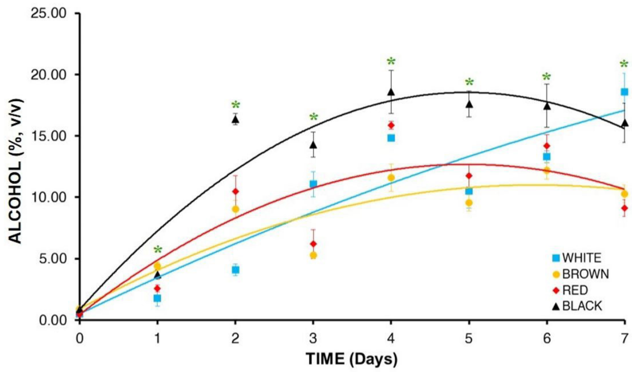

Figure 3. Alcohol content (%) of *Tapuy* made from different pigmented rice during the 7-day fermentation. Data points are the mean of 3 replicates. Error bars are the standard deviation for each data point. Lines are the best-fit curve using a 2nd order polynomial equation. There was an increase in the alcohol content of *Tapuy* made from different pigmented rice throughout fermentation. The final alcohol content of the different rice wines after fermentation was 9.13 to 18.58%. *Tapuy* made from *Ballatinao* black rice and *Bongkitan* white rice showed significantly higher ($p<0.01$) final alcohol content (16.07% and 18.58%, respectively) than red rice and brown rice (9.13% and 10.26%, respectively). *p-value <0.01.

Table 1. Total phenolic content (mg GAE/mL) of *Tapuy* made from different pigmented rice after the 7-day fermentation.

Rice Variety	Total Phenolics (mg GAE/mL)
White rice (*Bongkitan*)	$7.52^a \pm 0.04$
Brown rice (*Tinawon*)	$8.59^b \pm 0.04$
Red rice (*Kintoman*)	$9.08^c \pm 0.03$
Black rice (*Ballatinao*)	$9.73^d \pm 0.14$

Data values are the mean of 3 replicates ± standard deviation. *Tapuy* made from different pigmented rice exhibited a total phenolic content ranging from 7.52 to 9.73 mg GAE/mL after the 7-day fermentation. *Tapuy* made from black rice showed the highest total phenolic content (9.73 mg GAE/mL, $p<0.05$) among the *Tapuy* made from different pigmented rice. Mean values with different superscripts are significantly different at 5% level, as determined by Tukey's honest significant difference (HSD) test.

Rhizopus oryzae, Saccharomyces cerevisiae, Mucor rouxii) which are responsible for the increase in alcohol content of the *Tapuy* during fermentation (Banwa et al., 2020; Sanchez, 1999). *Tapuy* made from white rice and black rice showed a final alcohol content of 18.58% and 16.07%, respectively (Figure 3). These values are significantly higher ($p<0.01$) compared to the final alcohol content of *Tapuy* made from red rice and brown rice (9.13% & 10.26%, respectively). The higher alcohol content of *Tapuy* made from white rice and black rice, compared to *Tapuy* made from red rice and brown rice, was likely due to the larger number of fermentable saccharides found in glutinous rice, which were shown to produce higher alcohol content in rice wines (Coronel et al., 1981; Lai et al., 2019; Zhao et al., 2009).

3.3 Tapuy made from black rice had the highest total phenolic content

Phenolic compounds are diverse plant metabolites that have many health-promoting bioactivities, e.g. antimicrobial, anti-inflammatory, anti-thrombotic, and antioxidant activity (Shahidi & Ambigaipalan, 2015). The total phenolic contents of *Tapuy* made from different pigmented rice are shown in Table 1. The different rice wines exhibited a total phenolic content ranging from 7.52 to 9.73 mg GAE/mL after the 7-day fermentation. These values were significantly different ($p<0.05$) from each other with *Tapuy* made from white, brown, red, and black rice showing an increasing total phenolic content. This finding may be attributed to the varying amounts of anthocyanins in the different pigmented rice. Romero et al. (2012) showed that *Ballatinao* black rice had the highest total phenolic (9.30 mg GAE/g) and anthocyanin (5,674 mg/mL) contents compared to red rice (6.18 mg GAE/g & 60.9 mg/mL) and non-pigmented or white rice (1.03 mg GAE/g & 42.7 mg/mL). The total phenolic contents of the different rice wines in this study were 10-fold higher compared to the total phenolic content of commercial *Tapuy* from Benguet, Philippines (118-303 μg GAE/mL) and commercial 100% orange juice (0.77 mg GAE/mL), which is considered a healthy beverage (Hipol & Alma-in, 2018; Wern et al., 2016).

3.4 Tapuy made from black rice had the highest antioxidant activity

The DPPH radical scavenging activity of *Tapuy* made from different pigmented rice are shown in Figure 4. At day 0 of fermentation, black rice and red rice had DPPH radical scavenging activities of 25.88% and 22.27%, respectively, which were significantly higher ($p<0.01$) compared to the DPPH radical scavenging activities of white rice and brown rice (9.35% & 9.99%, respectively) (Figure 4). Walter et al. (2013) showed similar results where black and red rice had 8 to 14 times higher antioxidant activity compared to light brown rice. These differences in antioxidant activity were positively and significantly correlated ($R^2=0.9099$) with the total soluble phenolic compounds of the different pigmented rice (Goffman & Bergman, 2004; Walter et al., 2013). The higher levels of phenolic compounds in black rice (i.e., anthocyanins, particularly cyanidin-3-glucoside & peonidin-3-glucoside) and red rice (i.e., proanthocyanidins) may be responsible for their high antioxidant activities (Ghasemzadeh et al., 2018; Hou et al., 2013; Oki et al., 2002). As mentioned previously, *Ballatinao* black rice had the highest anthocyanin and total phenolic contents compared to red rice and non-pigmented or white rice, further suggesting the influence of anthocyanins and phenolic compounds on the antioxidant activity of black rice (Romero et al., 2012).

There was a significant increase ($p<0.01$) in the DPPH radical scavenging activity of the different *Tapuy* during the 7-day fermentation (Figure 4). The increase in antioxidant activity of the different *Tapuy* during fermentation may have been brought about by 2 possible mechanisms. First, the amount of available antioxidant compounds in the *Tapuy* may have increased through the action of starter culture microbial hydrolytic enzymes on the rice. Second, the alcohol produced during fermentation may have facilitated the extraction of antioxidant compounds in the rice. A study on the fermentation of *Haria*, an Indian fermented rice beverage, showed that the increase in antioxidant activity of this fermented rice beverage was related to the presence of higher amounts of oligosaccharide, phenolic compounds and flavonoids attributed to the action of hydrolytic enzymes from microorganisms during the course of fermentation (Ghosh et al., 2015). Apparently, this antioxidant activity increase is a common occurrence in fermentation, since it was also seen in several studies of fermentation of rice bran with *Rhizopus oryzae*, *L. lactic*, *L. plantarum*, *Aspergillus awamori*, and *Aspergillus oryzae* (Nisa et al., 2019; Oliveira et al., 2012; Shin et al., 2019). It is notable that these same microorganisms were also found in *Tapuy* fermented with *Bubod* (Sanchez, 1999). Another common microorganism in starter cultures, including *Bubod*, are yeasts. The production of alcohol by the action of yeast on rice starch may also contribute to the amount of phenolic compounds in rice wine by improving phenolic extraction during fermentation (Singkong, 2015).

The final DPPH radical scavenging activity of the different *Tapuy* were 45.44% to 70.63% (Figure 4). These values fall within the range of DPPH radical scavenging activity (50.0-88.5%) of commercial *Tapuy* from Benguet, Philippines (Hipol & Alma-in, 2018). *Tapuy* made from black rice exhibited the highest DPPH radical scavenging activity (70.63%, $p<0.01$) among the *Tapuy* made from different pigmented rice. This is consistent with the study by Koguchi et al. (2010), where they showed that rice wine made from purple/black rice had higher antioxidant activity compared to those made from ordinary brown rice and polished white rice. Similarly, Chay et al. (2020) also showed that Cambodian rice wine made from black sticky rice had higher antioxidant activity compared to those made from white sticky rice. There was a significant direct correlation between the DPPH radical scavenging activity and total phenolic content of the different *Tapuy* in this study ($r = 0.92$, $p<0.01$), suggesting that the high antioxidant activity of *Tapuy* made from *Ballatinao* black rice could be related to the high total phenolic and anthocyanin contents of this black rice variety. Previous studies of rice wines made from black rice showed that the high amounts of total phenolic compounds, including anthocyanins, found in black rice was related to the high antioxidant activity observed in the rice wines (Cai et al., 2019; Koguchi et al., 2010). The high anthocyanin content of *Ballatinao* black rice could also be responsible for the bioactivities in model organisms revealed from previous studies (Ona & Medina, 2015; Quebral & Medina, 2015; Velasco & Medina, 2014). Interestingly, the final DPPH radical scavenging activity of *Tapuy* made from brown rice (57.20%) was similar to the final DPPH radical scavenging activity of *Tapuy* made from red rice (57.66%) (Figure 4). Even though brown rice had a significantly lower antioxidant activity than red rice at the start of fermentation, the antioxidant activity of brown rice could have been enhanced during fermentation by the release of phenolic compounds (e.g. ferulic acid & *p*-coumaric acid) coming from the brown rice bran, which contains 70-90% of the total phenolic acid of the rice grain (Zhou et al., 2004).

3.5 Tapuy made from different pigmented rice have distinct sensory profiles

The sensory evaluations of *Tapuy* made from different pigmented rice are shown in Figure 5. Out of the six sensory

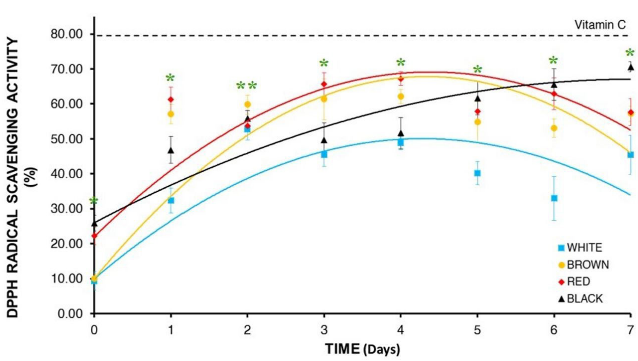

Figure 4. DPPH radical scavenging activity (%) of *Tapuy* made from different pigmented rice during the 7-day fermentation. Data points are the mean of 3 replicates. Error bars are the standard deviation for each data point. DPPH radical scavenging activity of the positive control (vitamin C) at 200 μg/mL is 79.97%. Lines are the best-fit curve using a 2^{nd} order polynomial equation. There was an increase in the DPPH radical scavenging activities of *Tapuy* made from different pigmented rice. At day 0, black rice and red rice already showed significantly higher ($p<0.01$) DPPH radical scavenging activities (25.88% and 22.27%, respectively) from brown rice and white rice (9.99% and 9.35%, respectively). At day 7, *Tapuy* made from black rice leveled-off at 70.63% DPPH radical scavenging activity; whereas *Tapuy* made from the other pigmented rice were declining. The final DPPH radical scavenging activities of the different rice wines after fermentation was 45.44% to 70.63%, where *Tapuy* made from black rice showed the highest DPPH radical scavenging activity at 70.63%. **p-value <0.05; *p-value <0.01.

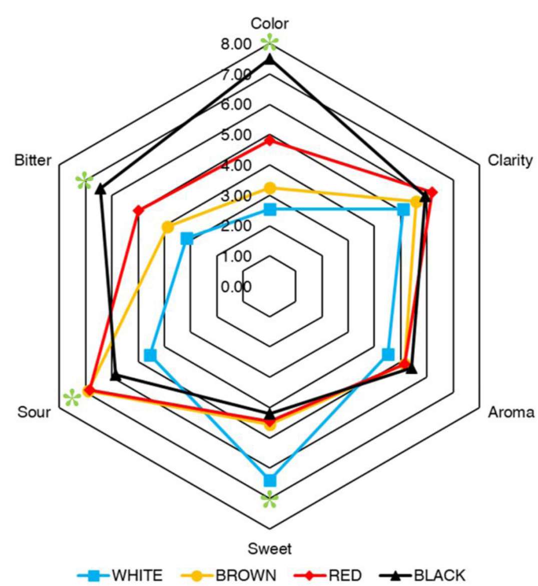

Figure 5. Sensory evaluation of *Tapuy* made from different pigmented rice after the 7-day fermentation. Data points represent the mean of each sensory attribute. All of the sensory attributes, except for aroma and clarity, showed significant difference ($p<0.05$) among the different rice wines. *Tapuy* made from black rice had the highest score on color (7.50) and bitterness (6.45) while *Tapuy* made from white rice had the highest score on sweetness (6.40). Means were compared by using Tukey's honest significant difference (HSD) test. *p-value <0.05.

attributes tested, i.e. color, sweetness, sourness, bitterness, clarity, and aroma, the last two did not show any significant difference (p>0.05) among the different rice wines. *Tapuy* made from black rice had the highest score for color and bitterness (7.50 & 6.45, respectively). The dark color and bitter taste of this rice wine may be attributed to the high anthocyanin and tannin contents, which is consistent with the observed high total phenolic content of the *Tapuy* made from this black rice variety (Table 1). Black rice had higher tannin and anthocyanin content in the bran layer compared to white rice (Bhattacharyya & Roy, 2018; Jun et al., 2012). In red wine, these phenolic compounds were found to be the main contributing factor to the bitter/astringent taste (Ma et al., 2014).

Tapuy made from white rice had the highest score for sweetness (6.40) among the different rice wines. The glutinous *Bongkitan* white rice variety, which provides more substrate for molds in the *Bubod* starter culture, saccharify into more simple sugars that produce a sweeter *Tapuy*. Lai et al. (2019) showed that rice wine made from glutinous rice had higher water-soluble sugars and was sweeter than rice wine made from non-glutinous rice.

4 Conclusions

This was the first study to describe the basic properties of *Tapuy* fermentation using different pigmented rice (black, red, brown and white) available in Benguet, Philippines. *Tapuy* made from *Ballatinao* black rice had the highest alcohol content, total phenolic content and antioxidant activity. Thus, *Tapuy* made with *Ballatinao* black rice may qualify as a health-promoting functional food which could positively impact the food industry. This *Tapuy* can increase the consumer market of indigenous rice wines since younger generation of consumers are more aware that nutrition is important in disease prevention. An increased demand for a healthier rice wine will increase production and sales of this indigenous beverage resulting to a greater demand for manpower among farmers that cultivate heirloom rice. Increase cultivation of this heirloom rice variety (*Ballatinao* black rice) will help preserve this heirloom rice against competition from cultivated commercial white rice. This will then maintain the unique genes found in this heirloom rice which could be used for the genetic improvement of common rice varieties in the future. Finally, this will also help preserve the Cordillera indigenous culture and traditions surrounding this heirloom rice. It is recommended that studies determining and documenting the beneficial bioactivities of *Tapuy* made from *Ballatinao* black rice be pursued in order to fully establish and promote this alcoholic beverage as a functional food product.

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