

Antioxidant potentials of indigenously produced Benguet *tapuy* (rice wine)

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Abstract

The objectives of this study were to evaluate the *in vitro* antioxidant capacity and measure the phenolic, flavonoid and tannin content of *tapuy*, the traditional Cordilleran rice wine (W) including its concentrate (WC) and volatile fraction (V). Two assays were used to assess antioxidant activity, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and the 2,2-azino-di-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay. The study evidenced antioxidant capacity for all wine samples in the two antioxidant assays with most WC exhibiting significantly higher ($p < 0.05$) antioxidant power. On the other hand, V had very limited radical quenching activities throughout the test. Furthermore, the total phenolic, total flavonoid and tannin concentrations were found in decreasing order: $WC > W > V$. The findings of this study indicate that *tapuy* contains natural bioactive compounds that could function as antioxidants i.e. phenolics, flavonoids and tannins. Also, the results of the DPPH and ABTS assays show that antioxidant property could be attributed to the hydrogen-donating capacity of such compounds. This study was able to establish the functionality of *tapuy* as a healthy beverage.

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Keywords

Antioxidant capacity
Phenolics
Flavonoids, Tannins
DPPH assay
ABTS assay

Introduction

Tapuy, the locally produced rice wine, is a traditional alcoholic drink in the northern part of the Philippines for more than 2000 years (Sakai and Caldo, 1985; Manaois and Morales, 2014). It is a sweet-acidic beverage that is produced by the saccharification and simultaneous yeast fermentation of roasted or steamed glutinous rice (Sanchez, 2008). This alcoholic drink has been intricately associated with the culture and traditions of the Cordillera people, including the Benguet Ibaloi tribe. It is consumed during special occasions such as weddings, fiestas, harvesting ceremonies and various cultural affairs (Bandonill *et al.*, 2009). Traditionally, the beverage is always prepared in the home and only in limited quantities; seldom for commercial purposes.

Asian neighbors have their own versions of rice wine which in terms of chemical production is actually rice beer (Aquino and Persoon, 2011). Rice-beer is a generic term referring to alcoholic beverages made from cereals, mainly rice, in East-Asia (Rhee *et al.*, 2011). These products are similar to *shaosinjju* of China, *sake* of Japan, *chongju* and *takju* of Korea, *tapuy* of Indonesia and *tapai pulul* of Malaysia (Shen *et al.*, 2011; Bhuyan *et al.*, 2014). *Sake* is almost similar to Chinese rice wine. They differ only in color; the Chinese version is reddish brown. In China, rice wine is not only consumed for drinking but also is

used as an ingredient in traditional medicine (Han and Xu, 2011). It has been claimed to have beneficial effects on the aging and on the prevention of cancer and cardiovascular diseases (Chen *et al.*, 2002). In a study by Yoshida *et al.* (2001), it was found that moderate consumption of the traditional Japanese rice wine, *sake*, may reduce cancer risk as a result of decreasing DNA damage in the body.

This popular Ibaloi alcoholic beverage commonly uses the Balatnao rice variety. In the Benguet method, this cooked rice is spread in a bamboo basket or bilao lined with banana leaves to cool. After cooling, powdered starter culture, *bubod*, is sprinkled on the surface of the cooled rice and then mixed thoroughly. The mixture is covered with fresh banana leaves and then placed in a warm place for three days after which the fermenting rice is transferred to an earthen jar and covered tightly. The mixture is allowed to ferment for two or more weeks (Sanchez, 2008). The juice formed is ladled out and consumed. In comparison, some *tapuy* makers in the Mt. Provinces and Ifugao add tobacco leaves and *Bidens pilosa* leaves to their starter cultures (Samonte, 2011).

Wines contain various bioactive components including polyphenolic compounds that are responsible for the quality of red wines, influencing their astringency, bitterness and color (Ghiselli *et al.*, 1998; Hosu *et al.*, 2014). These include such compounds as flavonoids, anthocyanins and

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tannins considered to have high antioxidant activity, protecting the body cells against oxidation (Bravo, 1998). In a study by Que *et al.* (2006), Chinese rice wine was found to contain various phenolic compounds with (+)-catechin and syringic acid being the dominant ones, which are highly correlated with the antioxidant activities in the rice wines that were tested. In addition, *sake*, made from purple rice wine, was found to have high phenolic and anthocyanic content which are compounds with high antioxidant properties (Teramoto *et al.*, 1994). In the Philippines, the commercial PhilRice wine made from red rice varieties has also been studied and was found to have comparable anthocyanin content to other types of pigmented wines such as red wine or blueberry wine (Bandonill *et al.*, 2009). Anthocyanins are polyphenolic compounds that have been found to exhibit four times greater antioxidant capacity than ascorbic acid (Zhang *et al.*, 2010).

Antioxidant compounds act by scavenging radicals known as reactive oxygen species (ROS). Cellular damage caused by ROS has been linked to lipid peroxidation and consequently to membrane injury, protein degradation, enzyme inactivation and disruption of DNA strands (Stadtman, 1990; Pietrini *et al.*, 2002; Bergamini *et al.*, 2004). The consumption of wine may help alleviate the effects of ROS and thus be ultimately beneficial for consumers (Que *et al.*, 2006; Bandonill *et al.*, 2009). Aside from those mentioned above, several clinical studies have demonstrated the health effects of wine in the diet including high antioxidant activity in blood serum (Whitehead *et al.*, 1995), decrease in the many types of cancer (Cao *et al.*, 1998), decrease in the incidence of cardiovascular diseases (Arranz *et al.*, 2012), increase in HDL (good) cholesterol with decrease in LDL (bad) cholesterol (Pal *et al.*, 2003) and decrease in the incidence of non-insulin dependent diabetes (Conigrave *et al.*, 2001).

It is already established that red and yellow wines do provide health benefits to those that consume them at moderate amounts (Que *et al.*, 2006). However, based on literature search, an in-depth study on Benguet *tapuy*'s antioxidant activities has not yet been conducted, specifically on those that have not been commercialized yet.

Rice wine production has already undergone commercialization here in the Philippines. Various institutions, including the Philippine Rice Research Institute (PhilRice) had efforts to standardize *tapuy* production for commercial purposes (Ablaza *et al.*, 2008; Bandonill *et al.*, 2009). However, the indigenous mode of rice wine production has been less studied in terms of their bioactive components.

In view of the above mentioned gaps, this study focused on evaluating the antioxidant activities of *tapuy* produced in different localities in Benguet. Moreover, the study aimed to investigate if the antioxidant components are present in the concentrate (WC) or distilled fraction (V) and to quantify the major bioactive components of samples (total phenolics, flavonoids and tannins) in order to explore the potential of *tapuy* as a beneficial fermented beverage.

Materials and Methods

Wine samples

Tapuy samples from seven Benguet localities (T1-Sablan; T2-Trinidad; T3-Atok; T4-Kapangan; T5-Bokod; T6-Tublay; T7-Tuba) were purchased/obtained from local native brewers as referred to by native folks. Sampling was similar to those of Hong *et al.* (2009) and Kim *et al.* (2014) where their wine samples were procured from local stores. These seven wine samples (W) were brewed using traditional Benguet method and were 15-21 days from the day of bottling (or final packaging). Three replicates for each were taken. The rice variety used for brewing was Balatinao rice (red rice). All samples were refrigerated at 5°C after collection.

The concentrate of the wine (WC) was obtained after it was concentrated to one-ninth of the original volume by a rotary evaporator at 30°C to remove the volatile compounds (Que *et al.*, 2006). The concentrate was then diluted nine times with distilled water before subjecting to chemical analyses. The volatile component (V) was collected after condensation. Samples collected were then subjected to the different antioxidant capacity assays and their phenolic, flavonoid, anthocyanin, and tannin content were quantified. Each test was also conducted in triplicate (n=3).

Biochemical properties

Wine samples were analyzed for their pH, alcohol content, total acidity (TA) and volatile acidity (VA). pH was measured using a bench pH meter (Eutech pH 700). Chemical parameters were determined according to standard methods of analysis (Bhuyan *et al.*, 2014). Alcohol content was measured by pycnometric method at 20°C and was expressed as % alcohol by volume. TA and VA contents were measured following the standard titration methods and expressed as g tartaric acid/L and g acetic acid/L, respectively.

Total antioxidant capacity assays (ABTS and DPPH assay)

Antioxidant activity was evaluated by the estimation of radical scavenging capacity using the ABTS test and the DPPH test, using three replicates for each test. For the ABTS experiment, the protocol of Thaipong *et al.* (2006) was used. Briefly, 6 ml each of 7.4 mM ABTS and 2.6 mM potassium persulfate in methanol was prepared. These solutions were mixed together and incubated at RT for 16 hr in the dark. This was then diluted with methanol until absorbance was at 1.1 ± 0.02 at 734 nm with methanol. From this solution, 1.9 ml was measured and was added to 1 ml of the sample to be tested. This was incubated for 2 hr at room temperature and the absorbance measured at 734 nm with methanol as blank. Antioxidant activity was compared with the standard Trolox. Calculation of antioxidant power as percentage inhibition was based on the formula of Spigno *et al.* (2007):

$$\% \text{ inhibition} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{ABTS}=0}} \right) \cdot 100$$

DPPH radical scavenging assay followed the protocol of Hipol (2014) with some modifications. For this experiment, 75 μl of the sample was added to 1.5 ml of the DPPH solution. The reaction mixture was incubated at 37°C for 30 mins. The absorbance was then measured at 517 nm with methanol as blank. Antioxidant activity was quantified using a standard curve of ascorbic acid and was also expressed in terms of % inhibition of the radical.

Determination total phenolic content

The assay was conducted based on the method of Hosu *et al.* (2014). Briefly, 1.5 mL of Folin–Ciocalteu reagent (0.2 mol/L) was added to 0.3 mL of each sample appropriately diluted with distilled water so as absorbance is between 0.200–0.800. The reaction mixture was allowed to react 5 mins and then, 1.2 mL of 0.7M Na_2CO_3 was added. All samples were incubated in the dark at RT for 120 mins, and the absorbance was measured at 760 nm. A calibration curve was obtained using gallic acid as standard and results were expressed as gallic acid equivalents (μg GAE/ml).

Determination total flavonoids content

The total flavonoids content was determined by the protocol of Hosu *et al.* (2014). Samples (0.5 mL) were treated with 0.4 mL of 25g/L AlCl_3 solution, 0.5 mL of 100 g/L CH_3COONa solution and 4 mL distilled water. After 15 mins, the absorbance of the mixture was measured at 430nm and the flavonoid content, expressed in catechin equivalents (μg catechin/mL

of sample) was calculated using the calibration curve obtained in the 0–400 $\mu\text{g}/\text{mL}$ concentration range.

Determination total tannins content

Tannin content was measured based on the protocol of Ribereau-Gayon *et al.* (2006) and Hosu *et al.* (2014). Two samples containing 4 mL of W or WC diluted ten times, 2 mL of distilled water and 6 mL of 12 N HCl. One of the tubes was heated at 100°C for 30 mins and 1mL of ethanol (95%) is added to solubilize the red color that appears. The other tube is not heated but also received 1 mL ethanol. Absorbances of all samples were measured at 470, 520 and 570 nm. The differences (ΔA) between the samples, measured at the same wavelength (ΔA_{470} , ΔA_{520} , ΔA_{570}), were calculated. Then, considering the ΔA_{470} , ΔA_{570} values, ΔA_{520} , values were calculated using:

$$\Delta A_{520} = 1:1 \times \Delta A_{470}$$

$$\Delta A_{520} = 1:54 \times \Delta A_{570}$$

Total tannins content expressed as g/L of wine was calculated as follows:

$$\text{TTC (g/L)} = 15.7 \times \text{minimum } (\Delta A_{520})$$

Statistical analysis

The normal distribution of the data was checked by Shapiro-Wilk test. One-way analysis of variance (ANOVA) and Tukey's honest significant differences was conducted using the Statistical Package for the Social Sciences (SPSS) version 17.0 at 5% level of significance.

Results and Discussion

pH and biochemical properties of tapuy wine

The samples were analyzed for their pH, alcohol content, total acidity and volatile acidity (Table 1). The samples were found to have a pH range of 3.01 to 3.74 which generally shows a weakly acidic pH. Lowest pH was observed in T1. Consistent with this, T1 also showed the highest TA and VA contents.

TA content was observed to be between 0.15 to 0.76 g tartaric acid per liter sample. As for volatile acidity measured as acetic acid content, the range was found to be from 0.210 g/L to 0.852 g/L. The results indicate significant variations in the W samples obtained. Similar observations were mentioned by Hong *et al.* (2009) where Korean *takju* chemical compositions, including pH, total sugar, ethanol, total acid and amino-nitrogen varied among *takju* wine samples.

According to Sanchez (2008), *tapuy* contains approximately 13-19% alcohol depending on the

Table 1. Mean values \pm SD (n = 3 replicates) of biochemical parameters in sample rice wines.

	pH	Alcohol (% Vol.)	Volatile Acidity	
			Total Acidity (g tartaric acid/L)	(g acetic acid/L)
T1	3.06 \pm 0.002 ^c	8.19 \pm 0.090 ^d	0.76 \pm 0.003 ^a	0.852 \pm 0.207 ^a
	3.55 \pm			
T2	0.005a ^b	19.83 \pm 0.550 ^a	0.25 \pm 0.005 ^c	0.287 \pm 0.021 ^b
		17.66 \pm		
T3	3.46 \pm 0.001 ^b	0.230 ^{ab}	0.30 \pm 0.018 ^c	0.272 \pm 0.002 ^b
T4	3.31 \pm 0.002 ^b	14.38 \pm 0.740 ^b	0.27 \pm 0.004 ^c	0.192 \pm 0.003 ^b
T5	3.74 \pm 0.005 ^a	12.71 \pm 0.310 ^c	0.42 \pm 0.008 ^b	0.123 \pm 0.040 ^b
T6	3.72 \pm 0.019 ^a	12.97 \pm 0.970 ^c	0.29 \pm 0.059 ^c	0.120 \pm 0.041 ^b
T7	3.52 \pm 0.017 ^{ab}	11.68 \pm 0.520 ^c	0.15 \pm 0.009 ^d	0.462 \pm 0.073 ^b

Means marked with different letters are significantly different ($p < 0.05$)

method employed, age of the wine, and the starter culture used. In this study where Benguet method of preparation was employed, mean alcohol content was 13.9%. Interestingly, the highest alcohol content was observed in T2 (19.83%) while the lowest was observed in T1 (8.19%) indicating a remarkable difference in alcohol content in the samples.

ABTS radical scavenging activity

In order to evaluate the antioxidant capacity of *tapuy*, two different antioxidant capacity assays were performed in this study. The test for antioxidant capacity involves various assay principles and experimental conditions. The ABTS and DPPH radicals are the two most extensively used and stable chromogen compounds for evaluation of the antioxidant activity of biological material (Yang *et al.*, 2009).

The ABTS assay is based on the generation of a blue/green ABTS^{•+} chromophore through the reaction between ABTS and potassium persulfate (Hosu *et al.*, 2014). When an antioxidant is introduced, the chromophore radical is reduced to stable ABTS. The method is known to be applicable to studies of both water-soluble and lipid-soluble antioxidants, pure compounds and food extracts (Re *et al.*, 1999). The results of the ABTS assay are shown in Figure 1. The highest antioxidant activity was found in WC of T7. All W and WC exhibited reduction of ABTS chromophore though the extent of antioxidant activity varied across the samples. We have noted WC had a significantly higher ($p < 0.05$)

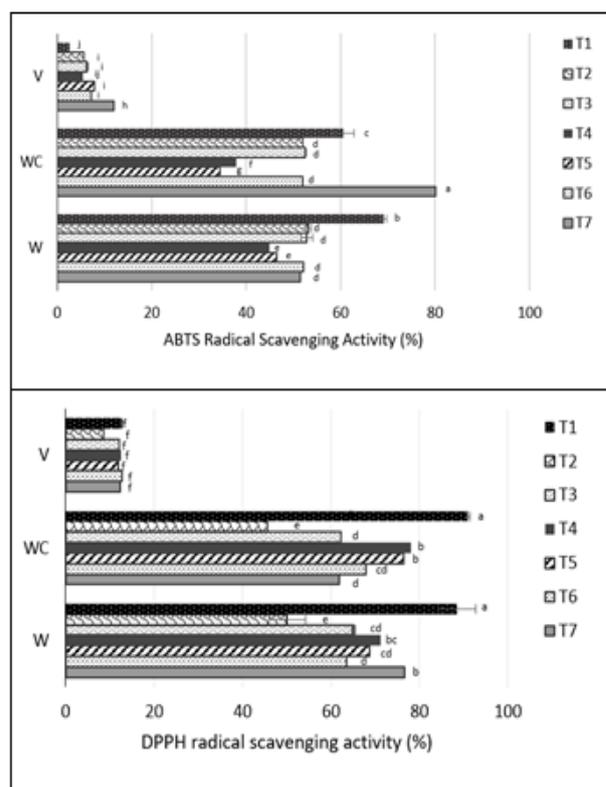


Figure 1. Scavenging activity of tapuy wines from the different municipalities of Benguet against a) ABTS radicals b) DPPH radicals. V – rice wine volatile fraction; WC – rice wine concentrate; W – rice wine. The bars represent the standard error (SE). Means marked with different letters are significantly different ($p < 0.05$).

radical activity than W samples in T7 while similar antioxidant activities were found in W and WC of T2, T3 and T6. Moreover, the antioxidant capacity of WC of T1, T4 and T5 were lesser than that of their corresponding W. Volatile component had limited ABTS antioxidant activity indicating that the antioxidant compounds are present both in the wine samples and in its concentrated fractions. In a similar study by Hong *et al.* (2009), the *takju* wine of Korea demonstrated a 32.08-61.99% antioxidant activity using ABTS assay. The *tapuy* samples in our study ranged 44.88-69.14%, showing a particularly higher value for our samples in Benguet.

DPPH radical scavenging activity

The DPPH radical scavenging activities of rice wine, its concentrate and volatile fraction are also shown in Figure 1. The action of antioxidant molecules on DPPH is mainly due to their hydrogen donating ability. In the presence of an antioxidant, the purple color typical to free DPPH radical decays (Hipol, 2014). The ability of *tapuy* to reduce DPPH was thus determined spectrophotometrically by the decrease in absorbance at 517 nm.

Both wine and WC exhibited significant

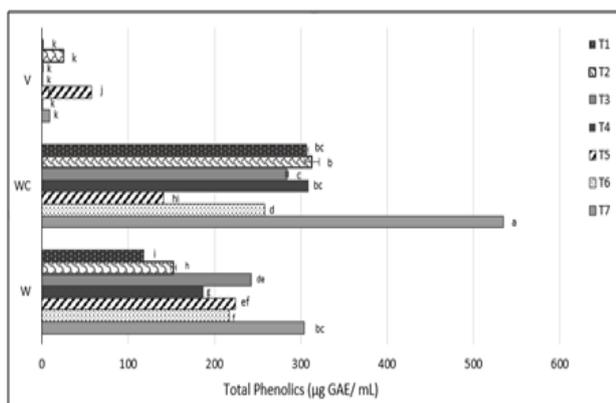


Figure 2. Total phenolic contents of tapuy rice wines from the different municipalities of Benguet in gallic acid equivalents $\mu\text{g GAE/mL}$. V – rice wine volatile fraction; WC – rice wine concentrate; W – rice wine. The bars represent the standard error (SE). Means marked with different letters are significantly different ($p < 0.05$).

scavenging of the free radical in all the samples, with T1 exhibiting highest DPPH radical scavenging activity. Our results show that *tapuy* wine and its concentrate evidence DPPH radical scavenging activity, with the following decreasing order: T-1 (88.5%) > T-7 (76.6%) > T-4 (71.3%) > T5 (68.6%) > T3 (65.1%) > T6 (63.6%) > T2 (50.0%). These data suggest that the antioxidant action of *tapuy* is most likely related to the hydrogen accepting capacity of its bioactive compounds. In comparison to the data of He *et al.* (2013), these values are somewhat lower to the 94.9% DPPH scavenging activity of Korean rice wine but still demonstrate effective reducing capacity.

The findings in the DPPH assay were very similar to the results of the ABTS assay. It was noteworthy that the wine and wine concentrate exhibited higher ($p < 0.05$) antioxidant activity in most instances while V had negligible antioxidant performance. Hence, it can be concluded that the antioxidant components are found mainly in the non-volatile fraction of the wine. Also, all W and WC exhibited practically similar DPPH antioxidant activity though the extent of antioxidant activity varied across the samples just like in the ABTS assay.

Total phenolics, total flavonoids and total tannins

Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic' as well as the ability to modify gene expression (Zhu *et al.*, 2000). Numerous epidemiological studies confirm significant relationship between the high dietary intake of phenolic flavonoids and the reduction of cardiovascular, carcinogenic and degenerative risks (Birt *et al.*, 2001; Brusselmans *et al.*, 2005). With the

numerous literatures confirming the health benefits of phenolics, the popularity of dietary intake of products rich in flavonoids and anthocyanins also surged (Podsedek, 2007). This includes consumption of wine products. While red wine is already established to possess high levels of polyphenols (Ghiselli *et al.*, 1998), a very limited number of studies have been carried out for rice wines.

Total phenolic content in *tapuy* ranged from 118-303 $\mu\text{g GAE/ml}$ (Figure 2). This is much higher than that of *takju* wine with 21.40 - 77.9 $\mu\text{g GAE/ml}$ (Hong *et al.*, 2009) but lower than that of North China rice wine with 722.43 $\mu\text{g GAE/ml}$ (He *et al.*, 2013). In addition, it was evident that higher amounts were found in the WC while significantly lower amounts were found in the volatile fractions ($p < 0.05$). Phenolic compounds act by inactivating lipid free radicals preventing decomposition of hydroperoxides into free radicals that may decrease the fluidity of cell membranes (Bacelar *et al.*, 2006; Ksouri *et al.*, 2007). A significant direct correlation ($p < 0.05$) between scavenging activity and the levels of polyphenols in the W samples was found i.e. $r = 0.85$ for the ABTS assay and $r = 0.77$ for the DPPH assay. Similarly, the total phenolic content in WC showed significantly positive correlations with antioxidant activities measured by ABTS ($r = 0.83$, $p < 0.05$) and DPPH ($r = 0.80$, $p < 0.05$) assays. These findings are in agreement with several studies that demonstrated polyphenols with high antioxidant capacity (Que *et al.*, 2006; Hosu *et al.*, 2014; Ivanova-Petropulos *et al.*, 2015). Phenolic compounds may therefore account for the antioxidant capacity of the *tapuy* samples. Moreover, it is hypothesized that the phenolic components in W and WC are derived mainly from the Balatnao rice (red rice) which is well known to possess polyphenolic compounds (Shen *et al.*, 2009).

On the other hand, total phenolic compounds were as little as 0.30 $\mu\text{g GAE}$ in every mL of the volatile fraction. This suggests that the phenolic components are not dissolved in the volatile fraction of the wine and instead is present in the non-volatile parts of W and WC. Hence, this most likely is the reason for the fairly low *in vitro* antioxidant capacity in V. These results are consistent with the findings in the two antioxidant capacity assays performed, ABTS and DPPH scavenging assays.

The results for the analysis of total flavonoids are shown in Figure 3. We noted flavonoid content ($\mu\text{g catechin per mL}$) in the wine samples (in decreasing order): T1 (270.9) > T5 (149.2) > T7 (148.7) > T4 (144.5) > T6 (137.0) > T2 (130.1) > T3 (124.2). Meanwhile, in the pairwise comparison of samples,

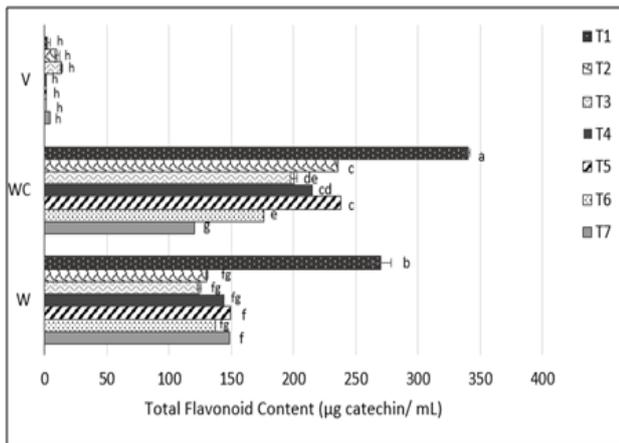


Figure 3. Total flavonoid contents of tapuy rice wines from the different municipalities of Benguet in catechin equivalents (μg catechin/mL). V – rice wine volatile fraction; WC – rice wine concentrate; W – rice wine. The bars represent the standard error (SE). Means marked with different letters are significantly different ($p < 0.05$).

V showed significantly lower amounts ($p < 0.05$) of flavonoids than the W and WC samples. On the other hand, the WC samples had markedly higher amounts of flavonoids from 140 to 176 μg catechin per mL.

Flavonoids are the largest and most important group of polyphenols having the capacity to act as antioxidants and protectant of the human body against reactive oxygen species (Birt *et al.*, 2001; Iyawe and Azih, 2011). These compounds have been proven to display a wide range of biochemical and pharmacological actions such as anticarcinogenic, antithrombotic, anti-inflammatory, and antimutagenic activities (Brusselmans *et al.*, 2005). In addition, flavonoids can act as free radical scavengers and terminate the radical chain of reactions that occurs during the oxidation of triglycerides in food system. Therefore, they are postulated to be effective antioxidant in oils, fats, and emulsions (Mustafa *et al.*, 2010). The consumption of wine including *tapuy*, is thus beneficial to consumers because of such health effects.

As illustrated in Figure 4, total tannin content ranged from 11.9 - 24.3 g per L of wine while it ranged from 26.7 to 43.5 per L for WC. Tannin content of V was close to zero (data not shown). In all cases, a significantly higher ($p < 0.05$) tannin content was observed in WC as compared to W samples. Tannins are bioactive compounds which are commonly measured in red wines manufactured from grapes. Such compound is usually referred to as tannic acid derivatives.

Although tannins are polyphenolic in nature, they possess specific bioactivities unique to them. Astringency of wine samples is mainly attributed to the tannin content. The amounts observed in this

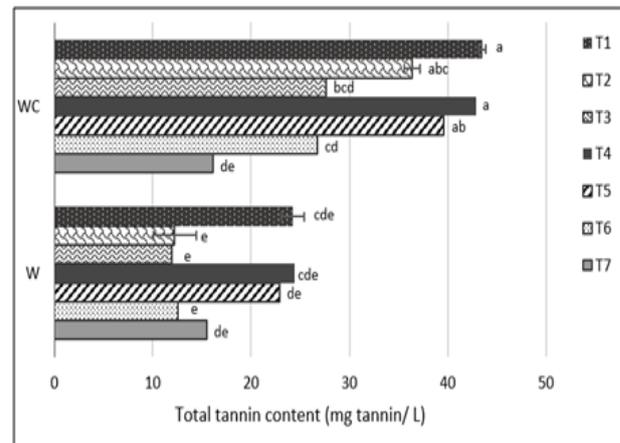


Figure 4. Total tannin contents of tapuy rice wines from the different municipalities of Benguet (mg tannin/L). WC – rice wine concentrate; W – rice wine. The bars represent the standard error (SE). Means marked with different letters are significantly different ($p < 0.05$).

study is lesser than that of red wines i.e. average of 544 mg tannin/L (Harbertson *et al.*, 2008; Mercurio *et al.*, 2010), hence, *tapuy* wine and its concentrate has less puckering taste and less astringent properties. Nonetheless, tannins are still established antioxidant compounds. The antioxidant activity of tannins is mainly attributed to their high reducing power which allows them to act as hydrogen donors and suppressor of hydroxyl radicals (Gulcin *et al.*, 2010).

In addition, correlation analyses were conducted. The ABTS scavenging activity showed a significant direct correlation with the total flavonoids in W ($r = 0.79$, $p < 0.05$) and in WC ($r = 0.82$, $p < 0.05$), as well as with the total tannins ($r = 0.77$, $p < 0.05$ and $r = 0.75$, $p < 0.05$, in W and WC, respectively). Similarly, the DPPH scavenging activity had a significant direct correlation with the total flavonoids ($r = 0.80$, $p < 0.05$ in W and $r = 0.82$, $p < 0.05$ in WC) likewise with the total tannins present ($r = 0.72$, $p < 0.05$ and $r = 0.78$, $p < 0.05$, in W and WC, respectively). All the V, on the other hand, had no significant correlations with the antioxidant activities. These data again very well corroborates previous studies that established the antioxidant activity of flavonoids and tannins (Gulcin *et al.*, 2010; Zhang *et al.*, 2016).

The above results evidence antioxidant activities in Benguet *tapuy* and the presence of antioxidant components. However, some limitations are worth noting. Although our hypotheses were supported statistically, the *tapuy* specific ingredients were not fully assessed. The differences in the raw materials may contribute to differences in the taste, quality and compositions of rice wine (Dung *et al.*, 2005; Hong *et al.*, 2009). In addition, several studies show that microbial count and different microbial species of

starter cultures contribute significantly to the physico-chemical properties of rice wine and fermentation efficiency (Chen and Xu, 2010; Palaniveloo and Vairappan, 2013; Bhuyan *et al.*, 2014). Though not studied in this research, it might be probable that these could have also contributed to the different qualities of the wine samples. Future work should therefore include a more detailed analysis of the ingredients used for *tapuy* making and investigate the correlation between these components and the antioxidant properties. Also, diversity of fungi and bacteria associated with *tapuy* are recommended to be investigated by culturing the starters in different selective and nutrient media.

Conclusion

This study was able to clearly demonstrate that *tapuy*, the traditional rice wine here in the Cordillera possess antioxidant properties. Results showed that W and WC had high radical scavenging activity while the volatile fraction exhibited very limited antioxidant capacity. Consistent with this, W and WC was found to possess significantly higher amounts of phenolic and flavonoid compounds, which are well established antioxidants. These results indicate that the rice wine produced here is nutritionally rich in terms of antioxidant compounds. Hence, this study was able to establish the beverage's healthful quality thereby possibly increasing consumer acceptance. Future works on elucidating the specific antioxidant compounds in *tapuy* wine are recommended.

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References

- Ablaza, M. J. C., Valerio, A. M., Mamucod, H. F., Bandonill, E. H. and Romero, M. V. 2008. Utilization of pure molds and yeast in the preparation of *tapuy* (rice wine). *Philippine Journal of Crop Science* 36 (2): 34-41.
- Aquino, D. M. and Persoon, G. A. 2011. Tradition and Change: Beer Consumption in Northeast Luzon, Philippines. In Schiefenhövel, W. and Macbeth, H. M. (Eds). *Liquid bread: Beer and brewing in cross-cultural perspective*, p. 197-208. New York: Berghahn Books.
- Arranz, S., Chiva-Blanch, G., Valderas-Martínez, P., Medina-Rejon, A., Lamuela-Raventós, R.M. and Estruch, R. 2012. Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. *Nutrients* 4 (7): 759-781.
- Bacelar, E. A., Santos, D. L., Gonc, B. C., Ferreira, H. F. and Correia, C. M. 2006. Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. *Plant Science* 170: 596-605.
- Bandonill, E. H., Morales, A. V., Ablaza, M. J., Mamucod, H. F. and Romero, M. V. 2009. Quality improvement and shelf-life enhancement of Philippine *tapuy* (rice wine). *Philippine Journal of Crop Science* 34 (1):12-18.
- Bergamini, C. M., Gambetti, S., Dondi, A. and Cervellati, C. 2004. Oxygen, reactive oxygen species and tissue damage. *Current Pharmaceutical Design* 10 (14): 1611-1626.
- Birt, D. F., Hendrich, S. and Wang, W. 2001. Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology and Therapeutics* 90 (2-3):157-177.
- Bravo, L. 1998. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. *Nutrition Reviews* 56 (11): 317-333.
- Brusselmans, K., Vrolix, R., Verhoeven, G. and Swinnen, J. V. 2005. Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. *Journal of Biological Chemistry* 280: 5636-5645.
- Bhuyan, D. J., Barooah, M. S., Bora, S. S. and Singaravadiel, K. 2014. Biochemical and nutritional analysis of rice beer of North East India. *Indian Journal of Traditional Knowledge* 13 (1): 142-148.
- Cao, G., Russell, R. M., Lischner, N. and Prior, R. L. 1998. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *The Journal of Nutrition* 128 (12): 2383-2390.
- Chen, C., Yin, Z. J. and Xu, S. 2002. The history and nutrition value of rice wine. *Liquor Making* 29: 55-56.
- Chen, S. and Xu, Y. 2010. The influence of yeast strains on the volatile flavour compounds of Chinese rice wine. *Journal of the Institute of Brewing and Distilling* 116: 190-196.
- Conigrave, K. M., Hu, B. F., Camargo, C. A., Stampfer, M. J., Willett, W. C. and Rimm, E. B. 2001. A prospective study of drinking patterns in relation to risk of type 2 diabetes among men. *Diabetes* 50 (10): 2390-2395.
- Dung, N. T., Rombouts, F. M. and Nout, M. J. 2005. Development of defined mixed-culture fungal fermentation starter granulate for controlled production of rice wine. *Innovative Food Science and Emerging Technologies* 6: 429-441.
- Ghiselli, A., Nardini, M., Baldi, A. and Scaccini, C. 1998. Antioxidant activity of different phenolic fractions separated from an Italian red wine. *Journal of Agricultural and Food Chemistry* 46 (2): 361-367.
- Gulcin, I., Huyut, Z., Elmastaş, M. and Aboul-Enein, H. Y. 2010. Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry* 3 (1): 43-53.
- Han, F. L. and Xu, Y. 2011. Identification of low molecular

- weight peptides in Chinese rice wine (Huang Jiu) by UPLC-ESI-MS/MS. *Journal of the Institute of Brewing* 117 (2): 238–250.
- Harbertson, J. F., Hodgins, R. E., Thurston, L. N., Schaffer, L. J., Reid, M. S., Landon, J. L., Ross, C. F. and Adams, D. O. 2008. Variability of tannin concentration in red wines. *American Journal of Enology and Viticulture* 59 (2): 210-214.
- He, S., Mao, X., Liu, P., Lin, H., Du, Z., Lu, N., Han, J. and Qiu, C. 2013. Research into the functional components and antioxidant activities of North China rice wine (Ji Mo Lao Jiu). *Food Science and Nutrition* 1 (4): 307-314.
- Hipol, R. 2014. Antioxidant potentials of culturable endophytic yeasts from *Phragmites australis* Cav. (Trin) ex Steud. from copper-contaminated mining site in Mankayan, Benguet. *Philippine Science Letters* 7 (2): 337-346.
- Hong, Y. H., Bae, S. H., Jung, E. Y., Son, H., Shin, K. S., Kwon, K. H. and Suh, H. J. 2009. Radical scavenging activities of Korean traditional rice wine, *takju*. *Journal of Food Science and Nutrition* 14: 109–115.
- Hosu, A., Cristea, V. M. and Cimpoiu, C. 2014. Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: prediction of antioxidant activities and classification of wines using artificial neural networks. *Food Chemistry* 150:113–118.
- Ivanova-Petropulos, V., Hermosin-Gutierrez, I., Boros, B., Stefova, M., Stafilov, T., Vojnoski, B., Dornyei, A. and Kilar, F. 2015. Phenolic compounds and antioxidant activity of Macedonian red wines. *Journal of Food Composition and Analysis* 41: 1-14.
- Iyawe, H. and Azihm, C. 2011. Total phenolic contents and lipid peroxidation potentials of some tropical antimicrobial plants. *European Journal of Medicinal Plants* 1 (2):33-39.
- Kim, M. K., Nam, P. W., Lee, S. J. and Lee, K. G. 2014. Antioxidant activities of volatile and non-volatile fractions of selected traditionally brewed Korean rice wines. *Journal of the Institute of Brewing* 120: 537-542.
- Ksouri, R., Megdiche, W., Debez, A., Falleh, H., Grignon, C. and Abdelly, C. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry* 45: 244–249.
- Leighton, F., Cuevas, A., Guasch, V., Perez, D. D., Strobel, P., San Martin, A. and Germain, A. 1998. Plasma polyphenols and antioxidants, oxidative DNA damage and endothelial function in a diet and wine intervention study in humans. *Drugs under Experimental and Clinical Research* 25 (23): 133-141.
- Manaois, R. V. and Morales, A. V. 2014. Evaluation of *tapuy* lees as a functional ingredient in the snack food polvoron. *Journal of Food Quality* 37: 196–202.
- Mercurio, M. D., Damberg, R. G., Cozzolino, D., Herderich, M. J. and Smith, P. A. 2010. Relationship between red wine grades and phenolics. 1. Tannin and total phenolics concentrations. *Journal of Agricultural and Food Chemistry* 58 (23): 12313-9.
- Mustafa, R. A., Hamid, A. A., Mohamed, S. and Abu Bakar, F. 2010. Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants. *Journal of Food Science* 75:28-35
- Pal, S., Ho, N., Santos, C., Dubois, P., Mamo, J., Croft, K. and Allister, E. 2003. Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of ApoB100 from human HepG2 cells. *The Journal of Nutrition* 133 (3): 700-706.
- Palaniveloo, K. and Vairappan, C. S. 2013. Biochemical properties of rice wine produced from three different starter cultures. *Journal of Tropical Biology and Conservation* 10: 31–41.
- Philippine Rice Research Institute. 2011. *PhilRice tapuy (Rice Wine) cookbook and cocktails: A collection of recipes and cocktails featuring Philippine rice wine*. Nueva Ecija: PhilRice Publishing.
- Pietrini, F., Iannelli, M. A. and Massacci, A. 2002. Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. *Plant, Cell and Environment* 25: 1251-1259.
- Podsedek, A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *Lebensmittel-Wissenschaft und Technologie- Food Science and Technology*. 40:1–11.
- Que, F., Mao, L. and Pan, X. 2006. Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. *Food Research International* 39: 581–587.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26: 9-10.
- Ribereau-Gayon, P., Glories, Y., Maujean, A. and Dubordieu, D. 2006. *Handbook of Enology*. Chichester: John Wiley.
- Rhee, S. J., Lee, J. E. and Lee, C. H. 2011. Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories* 10 (1): S5.
- Sakai, H. and Caldo, G. A. 1985. Microbiological and chemical changes in *tapuy* fermentation. *Journal of Fermentation Technology* 63:11-16.
- Samonte, C. 2011. *Physico-chemical Properties of Rice Wine as Influenced by the Formulation of Indigenous Yeast (Bubod)*. Benguet, Philippines: Benguet State University, MA thesis.
- Sanchez, P. C. 2008. *Rice wine (Tapuy)*. In *Philippine Fermented Foods: Principles and Technology*, p. 97-121. Quezon City: University of the Philippines Press.
- Shen, Y., Jin, L., Xiao, P., Lu, Y. and Bao, J. 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *Journal of Cereal Science* 49 (1): 106-111.
- Shen, F., Ying, Y., Li, B., Zheng, Y. and Hu, J. 2011. Prediction of sugars and acids in Chinese rice wine by mid-infrared spectroscopy. *Food Research*

International 44: 1521–1527.

- Spigno, G., Tramelli, L. and De Faveri, D. M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering* 81 (1): 200–208.
- Stadtman, E. R. 1990. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. *Free Radical Biology and Medicine* 9 (4): 315-325.
- Teramoto, Y., Saigusa, N., Ueda, S. and Yoshizawa, K. 1994. Production and characteristics of red rice *sake*. *Journal of the Institute of Brewing* 100: 3-6.
- Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19: 669–675.
- Whitehead, T. P., Robinson, D., Allaway, S., Syms, J. and Hale, A. 1995. Effect of red wine ingestion on the antioxidant capacity of serum. *Clinical Chemistry* 41 (1): 32-35.
- Yang, H. H., Song, H. B., Eun, Y. J., Heung-Soo, S., Kwang, S. S., Ki, H. K. and Hyung, J. S. 2009. Radical scavenging activities of Korean traditional rice wine, *takju*. *Preventive Nutrition and Food Science* 14 (2): 109-115.
- Yoshida, R., Shioji, I., Kishida, A. and Ogawa, Y. 2001. Moderate alcohol consumption reduces urinary 8-hydroxydeoxyguanosine by inducing of uric acid. *Industrial Health* 39: 322–329.
- Zhang, K., Yu, H., Shi, K., Zhou, Y., Yu, J. and Xia, X. 2010. Photoprotective roles of anthocyanins in *Begonia semperflorens*. *Plant Science* 179: 202–208.
- Zhang, S. L., Peng, D., Xu, Y. C., Lu, S. W. and Wang, J. J. 2016. Quantification and analysis of anthocyanin and flavonoids compositions, and antioxidant activities in onions with three different colors. *Journal of Integrative Agriculture* 15 (9): 2175-2181.
- Zhu, Q. Y., Huang, Y. and Chen, Z. Y. 2000. Interaction between flavonoids and α -tocopherol in human low density lipoprotein. *Journal of Nutritional Biochemistry* 11: 14–21.