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Towards the Establishment of Baseline Scientific Information Based on the Volatile Organic Compounds (VOCs) of Philippine Traditional Alcoholic Beverages

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Investigations on four Philippine traditional alcoholic beverages – namely, *lambanog, tapuy*, *basi*, and *tuba* – were carried out by determining volatile organic compounds (VOCs) in these beverages. Headspace gas chromatography with flame ionization detection (HS-GC-FID) is employed in this study to quantify four compounds – namely, ethanol, ethyl acetate, isoamyl alcohol, and isobutyl alcohol. Mean concentration values for the these VOCs within each beverage type were found to be as follows: 35.69% (v/v) ethanol, 329.67 mg/L ethyl acetate, 145.03 mg/L isoamyl alcohol, and 51.86 mg/L isobutyl alcohol for *lambanog*; 10.86% (v/v) ethanol, 212.87 mg/L ethyl acetate, 175.82 mg/L isoamyl alcohol, and 37.98 mg/L isobutyl alcohol for *tapuy*; 11.06% (v/v) ethanol, 114.18 mg/L ethyl acetate, 247.45 mg/L isoamyl alcohol, and 66.54 mg/L isobutyl alcohol for *basi*; and 8.79% (v/v) ethanol, 351.77 mg/L ethyl acetate, 68.74 mg/L isoamyl alcohol, and 16.23 mg/L isobutyl alcohol for *tuba*. Results showed that there is wide variability in the VOCs quantified in the four beverages, possibly on account of the sampling site, raw material used, and manufacturing process. Preliminary gas chromatography – mass spectrometry (GC-MS) studies qualitatively confirm the presence of other families of VOCs. The results from this study are envisioned to serve as new baseline information on Philippine traditional beverages.

Keywords: *basi*, Philippine traditional alcoholic beverages, *lambanog*, *tapuy*, *tuba*, VOCs

INTRODUCTION

Many traditional alcoholic beverages derived from local or endemic plants can be found in the different regions of the Philippines. Among the more popular alcoholic beverages are *lambanog*, *tapuy*, *basi*, and *tuba*. *Lambanog* is a distilled spirit derived from the fermented sap of palm trees called *tuba*. The sap is sourced from palm trees such as coconut palm (*Cocos nucifera*), nipa palm (*Nypa fruticans*), sugar palm (*Arenga pinnata*), or *buri* palm (*Corypha utan Lam*.).

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Lambanog is characterized by its slight fruity smell. Despite the distillation process it undergoes, it retains distinct flavors and aromas of its starting material (Sanchez 2008). *Lambanog* is a staple alcoholic beverage in some provinces in Luzon such as Laguna, Batangas, and Quezon provinces.

Tapuy or rice wine, obtained from fermented rice, is widely enjoyed in the Cordillera Administrative Region (CAR), a mountainous region north of Luzon. *Tapuy* is sweet and acidic, is cloudy but is also available in the clarified form. A starter culture (*bubod*) is used for the fermentation process of *tapuy* (Sanchez 2008).

Basi or sugarcane wine, derived from fermented sugar cane juice, is a popular beverage in some provinces in Northern Luzon such as Ilocos Norte, Ilocos Sur, La Union, and Pangasinan. *Basi* is sweet with slight sour notes and slightly turbid. Its color varies from dark red to a golden-brown hue. It is manufactured through two distinct processes based on existing practice in La Union, Pangasinan, and provinces in the Ilocos region. These methods differ from each other depending on the type of starter culture used for the fermentation of boiled sugar, length of the fermentation period, and manner of storage (Sanchez 2008).

Tuba or palm wine, sometimes known as coconut toddy, is consumed extensively in the Visayas, located in the central part of the Philippine archipelago. *Tuba,* is also popular in Quezon province and in the Bicol region. It is oyster-white in color and is sweet, with a hint of sourness that intensifies with time.

The typical ethanol content of *lambanog* ranges from 30–40%*,* 7–16% in *tapuy,* 10–16% in *basi*, and 10–13% in *tuba*. The Philippines' Food and Drug Administration (FDA) and the Bureau of Product Standards (BPS) under the Department of Trade and Industry, have set standards for the wine and alcohol industry. In particular, the standards specify that *basi* must contain a minimum of 12%(v/v) and, for *lambanog*, a minimum of 30%(v/v) ethanol (BPS 2009, 2011). Presently, there are no standards for *tapuy* and *tuba.*

To date, there are no standard methods for quality control for these alcoholic beverages, nor are there systematic determinations of the exact alcohol content of these said beverages. Some local makers engaged in medium-scale production of *lambanog* utilize an alcohol hydrometer to establish the approximate alcohol content (Limpe-Aw 2015, pers. comm.). Among the limited works that concern *lambanog*, *tapuy*, *basi*, and *tuba* is that of Sanchez (2008). It focuses on the principles and technology of fermentation of various foods and beverages. Investigations on the organic acid content of fresh *tuba* and coconut toddy as a function of the fermentation period have been carried out (Fernandez and Carandang 1990). Studies on the improvement of the shelf-life of *tapuy* by optimizing process parameters have also been reported (Bandonill *et al.* 2009). Orden *et al.* (2015) undertook characterization studies of wine parameters of selected locally produced wine in the Bicol region, including *lambanog* and *tuba*, with the purpose of confirming the compliance of these beverages to the Philippine National Standards for distilled fermented coconut sap (BPS 2011) and tropical wines (BPS 2010). None of these works, however, specifically involved systematic queries on the other chemical compounds or VOCs present in these traditional beverages.

In other countries, there are many scientific investigations on traditional alcoholic beverages. For instance, in Taiwan, the chemical quality during fermentation of a sugarcane wine similar to *basi* was examined on the basis of sugar, ethanol, volatile compounds, and other chemical properties (Tzeng *et al.* 2010). Thailand's fermented rice wine called *ou*, *sato*, or *krachae* – comparable to *tapuy* – has been profiled for its volatile flavor compounds using GC-FID after extraction of volatiles through conventional distillation techniques (Sirisantimethakom 2008), HS-GC-FID, and GC – olfactometry techniques (Chuenchomrat *et al.* 2008). Another study on *krachae* correlated its VOCs to the nitrogen content of the starting materials used such as glutinous rice and wheat rice (Amatayakul *et al.* 2012). Malaysia's rice wine called *lihing* has been studied for its biochemical and chemical properties (Palaniveloo and Vairappan 2013). *Makgeoli*, a Korean rice wine, has been extensively investigated for its chemical and sensory profiles based on descriptive, chemical, and volatile compound analyses (Jung *et al.* 2014). The changes in the aroma compounds during aging of the Japanese traditional rice wine, *sake*, have been studied using aroma extract dilution analysis and stir bar adsorptive method (Isogai *et al.* 2005). A beverage in Malaysia – similar to *tuba*, called *air nira* – was studied for its volatile compounds in its fresh and fermented states (Nur Aimi *et al.* 2013). The study established the differences in the VOCs types between fresh and fermented *air nira*. Meanwhile, a palm wine called *nsamba* in Congo was subjected to GC-MS wherein fifteen volatile compounds in the wine were identified (Dhellot *et al.* 2014). *Mezcal* of Mexico, comparable to *lambanog*, has been subjected to headspace GC for the determination of organic compounds as a function of the agave species used (Arellano *et al.* 2012). *Ogogoro* – a traditional Nigerian distilled beverage derived from palm wine, also similar to *lambanog* – has been subjected to physicochemical investigations (Adeleke and Abiodun 2010).

Admittedly, there is a distinct lack of scientific literature on Philippines traditional beverages. This study aims to fill this research gap by establishing the levels of selected VOCs that are easily accessible from the perspective of analytical determination. The results from this study are envisioned to serve as baseline information for these Philippine traditional beverages. This is the first time that new information on these beverages will be available, which can have an enormous impact on future product quality improvement, better market competitiveness of Philippine traditional alcoholic beverages, and – above all – safeguarding human health. This information, hopefully, also reawaken new appreciation among consumers for these traditional beverages. Lastly, this study also aims to provide data input to future policy formulations that will impact on the manufacture, sale, and consumption of alcoholic beverages that may empower local makers

of traditional alcoholic beverages to expand its market locally and internationally.

MATERIALS AND METHODS

Reagents and Solutions

All reagents used were purchased commercially and were of the highest grade available: absolute ethanol (Merck 1.00983.25000), ethyl acetate (J.T. Baker, Baker Analyzed A. C.S. Reagent 9280-3), propan-1-ol (RCl Labscan AR1161-G4L), isobutyl alcohol (J.T. Baker, Baker Analyzed A.C.S. Reagent 9044-03), isoamyl alcohol (J.T. Baker, Baker Analyzed A.C.S. Reagent 9038-01), and acetonitrile (J.T. Baker, Baker Analyzed A.C.S. Reagent 9152-80). The standard solutions used for the quantification of the respective VOCs in the alcoholic beverage samples contained acetonitrile as internal standard (IS). High purity water was used in the preparation of standards and samples.

Samples

A total of 36 samples with the following distribution: seven *lambanog* samples, 10 *tapuy* samples, 10 *basi* samples, and nine *tuba* samples were used in the study. Except for three *lambanog* samples provided by the manufacturer, all *lambanog* samples were purchased from commercial sources. *Tapuy* samples were all from the CAR. Most of the samples were bought from public markets and small retailers. *Basi* samples were from Pangasinan, La Union, Ilocos Norte, and Ilocos Sur. *Tuba* samples were from Quezon Province, Bicol Region, and the Visayas.

HS-GC-FID Analysis

VOCs were determined using static HS-GC-FID. All samples were analyzed without any prior sample treatment. Five hundred microliters (500 µL) of the standards and the samples were accurately measured into 20-mL clear headspace vials with aluminum seal caps fitted with silicone/ PTFE septa. The analysis of ethanol was carried out separately due to its higher occurrence compared to the other VOCs in the samples. Three replicate trials were carried out for the determination of the VOCs. For this purpose, 1.5-mL aliquots of *lambanog* samples were used. Meanwhile, for *tapuy*, *basi*, and *tuba*, 3.0-mL aliquots were used. For the four beverages, sample aliquots used were as follows: ethyl acetate determination (3.0–10.0 mL), and isoamyl alcohol and isobutyl alcohol determination (5.0–10.0 mL). All sample aliquots were diluted to a final volume of 25.0 mL, with IS added prior to chromatographic analysis.

The identity of the selected VOCs in the samples was verified by determining their relative retention times (RRT) with respect to an IS. The RRTs of the VOCs in the sample were then matched with established RRTs of the pure VOC. A mixture of pure VOC standards – namely, ethanol, ethyl acetate, isoamyl alcohol, active amyl alcohol, n-propanol, methanol, acetaldehyde, isobutyl alcohol, and acetonitrile – was prepared by combining 200 µL of each AR grade VOC. This mixture was subsequently diluted to 25 mL with high purity water. Five hundred microliters (500 µL) of this standard mixture was analyzed using HS-GC-FID. The RRT of each VOC against the IS (acetonitrile) was established by taking the ratio of the retention time of each VOC to the retention time of the IS.

A Shimadzu gas chromatograph GC-14B equipped with an FID and fitted with a headspace autosampler accessory AOC-5000 (Shimadzu, Japan) was used. The injection volume was set to 250 µL. Syringe temperature was 75 °C and syringe flush time 30 s. The incubation temperature of the agitator was set at 75 °C. Headspace extraction and vial shaking were set to 10 min with 10 s agitation and 30 s rest. Agitator speed was 500 rpm and GC cycle time, including oven cooling time, was 19 min.

The separation was achieved using a Supelco SPB™ 1000 capillary column 30 m x 0.25 mm x 0.25 µm film thickness. GC oven was set at 40 °C for 1 min, then increased to 42 °C at 1 °C/min, held for 2 min and finally increased to 122 °C at 20 °C/min, held for 5 min with a linear nitrogen flow of 2.5 mL/min. Injector temperature was set at 150 °C and the detector was set at 250 °C.

Evaluation of Performance Characteristics of the Method

For the validation studies, figures of merit such as accuracy or trueness, precision, linearity, limits of detection (LOD) and quantitation (LOQ) were established in accordance with Eurachem guidelines (Magnusson and Örnemark 2014). Due to the unavailability of certified reference materials, the accuracy of the method was evaluated by carrying out recovery studies. Low and high levels of each VOC standard were spiked to one of the *lambanog* samples. The levels of spiked standards were 1% for ethanol, 24 mg/L and 1100 mg/L for both ethyl acetate and isoamyl alcohol, and 5.2 mg/L and 450 mg/L for isobutyl alcohol. For ethanol, being a major constituent, the low-level spike was no longer carried out. Seven replicate determinations were carried out for each level of spike per VOC. Precision was established from triplicate measurements for each sample. The linear range was derived from the calibration curves by preparing a series of analyte standards. Acetonitrile, as the IS, was added to both standards and samples. Peak area ratio of the analyte to the IS was plotted against the concentration of the analyte. The LOD was estimated using y-residuals

of the regression line to obtain $s_{y/x}$, which is equivalent to the standard deviation of the blank (s_B). Employing the calculated intercept from the regression analysis, as an estimate of the reading of the blank, the LOD for the determination (y_B) was calculated.

Preliminary Qualitative Analysis Using GC-MS

Qualitative identification of other VOCs present in two samples from each beverage type was carried out by GC-MS analysis. The analysis was performed without any prior sample treatment using a Shimadzu GCMS-QP2010 Ultra™ gas chromatograph-mass spectrometer with high-performance quadrupole mass analyzer. Injection of the pure sample was carried out using AOC-20i/s™ automatic liquid sample injection system. The sampling syringe and needle were rinsed with the sample four times prior to the injection of a 0.1-µL sample. The plunger suction and injection, and syringe insertion speed were set to "high." The injection mode was normal with an injection port dwell time of 0.3 s.

The separation was achieved using a Supelcowax10 (30.0 m x 0.32 mm x 0.25 µm) capillary column. The GC oven was set at 35 °C for 3 min then increased to 180 °C at 10 °C/min, being held for 10 min; this was finally increased to 240 °C at 5 °C/min, being held for 2.50 min, with a linear nitrogen flow of 1.57 mL/min. The injector port was heated at 220 °C with a split ratio of 20. Further, high sensitivity detection was achieved using a quadrupole mass spectrometer equipped with an electron impact ionization source. The interface and ion source were maintained at 240 °C and 220 °C, respectively. Detection of ions with mass-to-charge ratio (m/z) of 45–500 started at 4.50 min to 42.00 min with a scan speed of 1666 and a detector gain of 0.00kV. Due to the high concentration of ethanol in the samples, MS detection only started after its elution at 4.50 min (solvent cut time). Ultimately, post-run analysis and peak identification were performed using the Shimadzu GCMS Solutions™ software, NIST11, and Wiley7 libraries.

Statistical Analysis

Linear regression was carried out using Microsoft Excel. For each beverage, significant differences in mean concentrations of VOCs were tested by one-way analysis of variance (ANOVA). Tukey's test was used for *post hoc* comparison. Statistical treatment was carried out using the R software, an open-source software for programming language and statistical computing and graphics. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Selection of VOCs for Quantitative Determination

The RRTs of pure VOCs acquired from the HS-GC-FID method using the mixture of pure VOC standards consisting of ethanol, ethyl acetate, isoamyl alcohol, active amyl alcohol, *n*-propanol, methanol, acetaldehyde, isobutyl alcohol, and acetonitrile were compared to the RRTs of peaks obtained for *lambanog*, *tapuy*, *basi*, and *tuba* samples. Four appreciable signals were observed from the chromatograms of these beverages, which corresponded to ethanol, ethyl acetate, isoamyl alcohol, and isobutyl alcohol. For some samples, a few other peaks were observed within the chromatographic run (14 min) but the signal-to-noise ratios for these peaks were no longer appreciable. Extending the chromatographic run was also attempted to elute other possible compounds, but sample throughput was greatly sacrificed and no other peaks were detected.

Based on this, the VOCs ethanol, ethyl acetate, isoamyl alcohol, and isobutyl alcohol were selected since these are the most common compounds present in alcoholic beverages reported in the literature (Chen *et al*. 2013; Chuenchomrat *et al.* 2008; Jung *et al*. 2014). Further, these VOCs exhibited good signal-to-noise ratios that are desirable from the analytical point of view. Figure 1 shows typical chromatograms of *lambanog*, *tapuy*, *basi*, and *tuba*.

Method Validation

The HS-GC-FID method employed in this study was initially validated to ensure fitness for its purpose. Performance characteristics such as linearity, accuracy, precision, and the LOD and LOQ of the HS-GC-FID method used in this study are shown in Table 1. Linearity between peak area ratio of the VOC to the IS and concentration of the standards for each VOC has been found to be excellent. Calibration carried out in the concentration range of 0.5–3.0% ethyl alcohol consistently gave linear plots. Analogously, linear plots were obtained for ethyl acetate (25–1100 mg/L), isobutyl alcohol (10–500 mg/L range), and isoamyl alcohol (25–1100 mg/L range). The coefficients of determination reported in Table 1 are average values based on several calibrations.

Good recovery values were obtained for seven replicate trials for each low spike and high spike concentration levels. While the generally acceptable range for recovery is from 80–120%, it must be stressed that – for analytes in the parts per million level (mg/L) – a satisfactory percent recovery must be between 60–115% (Kocourek 2012). Based on the values seen in Table 1, the HS-GC-FID method provides very good recoveries for the selected VOCs.

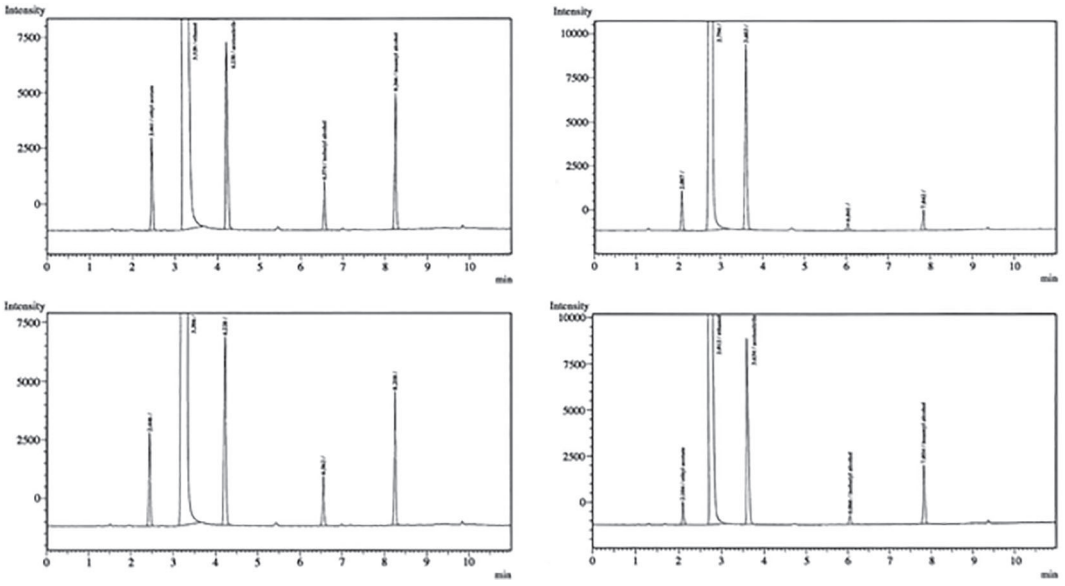

Figure 1. Chromatograms of *lambanog* (L05; upper left), *tapuy* (Y08; upper right), *basi* (B04; lower left), and *tuba* (T04; lower right). Peaks from left to right: ethyl acetate, ethanol, acetonitrile (IS), isobutyl alcohol, and isoamyl alcohol.

Table 1. Figures of merit for HS-GC-FID method.

Figure of merit		Ethanol	Ethyl acetate	Isoamyl alcohol	Isobutyl alcohol
Trueness (% recovery)	Low spike	[a]	88.70%	88.20%	98.60%
	High spike	95.50%	95.30%	98.90%	86.80%
Precision (% RSD range)		0.20–14%	0.70–14 %	0.20–18 %	0.40–9.0%
Linearity (R^2)		0.999	0.997	0.998	0.996
Limit of detection (LOD)		0.001% (10 mg/L)	5 mg/L	2 mg/L	2 mg/L
Limit of quantitation (LOQ)		0.004% (40 mg/L)	20 mg/L	7 mg/L	6 mg/L

[a]Not carried out

The precision of the method is very satisfactory based on the RSD values for the replicate measurements of the VOCs in the samples. These can be found in Tables I–IV of the Appendices section. The RSD values of the replicate measurements did not exceed the expected precision as estimated by the Horwitz criterion (Horwitz 1983). Meanwhile, the LODs of the HS-GC-FID method for each VOC are also quite good. The very satisfactory performance of the HS-GC method based on the validation results demonstrates the applicability of the method for the quantification of the selected VOCs in the four traditional alcoholic beverages under study.

Quantification of Selected VOCs in Samples

Due to the high occurrence of ethanol in the samples, quantification of this VOC was carried out separately. A 2.5- to 5-fold dilution was used for this purpose. The mean concentration of ethanol (%v/v), ethyl acetate (mg/L), and isoamyl and isobutyl alcohols (mg/L) can be seen in Figure 2.

The corresponding values of the standard error of the mean (SEM) for each VOC can be found in Appendix Table V. There are noticeable high SEM values for some VOCs in each beverage type. Ideally, a smaller SEM value is desirable to have an accurate approximation of a population mean of these VOCs in the four beverages. Increasing the number of samples per beverage can certainly improve SEM values.

Meanwhile, Figure 3 indicates the dispersion of the concentrations of every VOC per sample within a beverage type.

Ethanol content (Figure 3; upper left) in *basi* samples has the lowest variability in contrast to the three other beverages. The variability of this VOC in *tapuy* and *tuba*

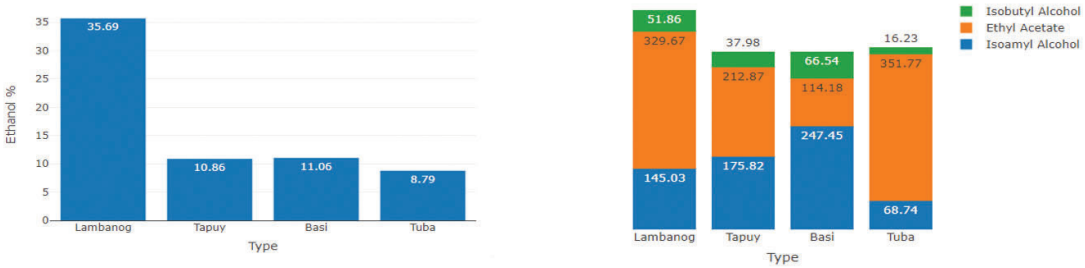

Figure 2. Left hand side: mean values of ethanol content in % (v/v). Right hand side: mean values of ethyl acetate, isoamyl alcohol, and isobutyl alcohol concentrations in mg/L of *lambanog* (n = 7), *tapuy* (n = 10), *basi* (n = 10), and *tuba* (n = 9).

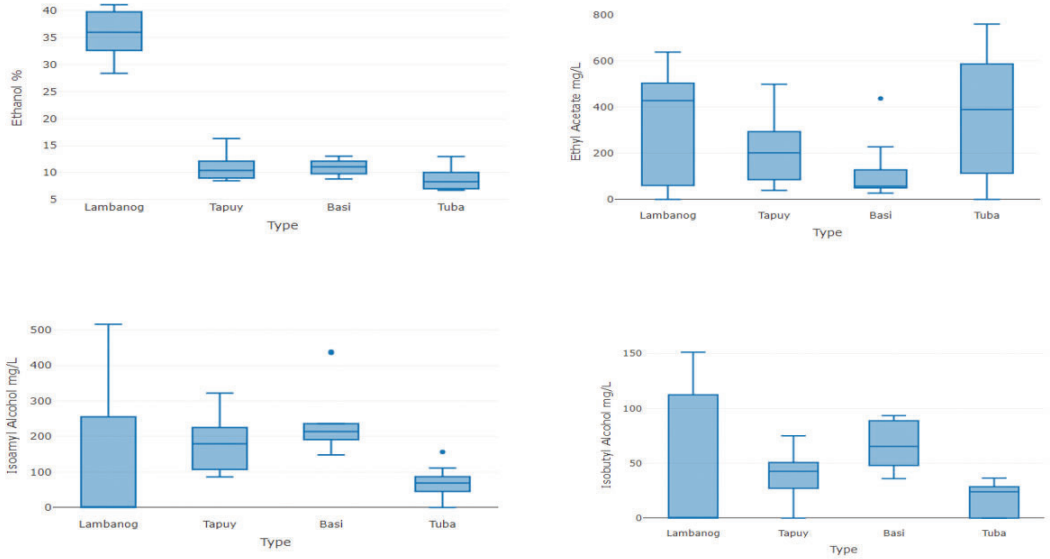

Figure 3. Box and whisker plots for ethanol (upper left), ethyl acetate (upper right), isoamyl alcohol (bottom left), and isobutyl alcohol (bottom right) in *lambanog*, *tapuy*, *basi*, and *tuba.*

samples appears to be similar in trend but the spread in the *lambanog* samples is more pronounced, with almost half of the samples clustering around the median. In Figure 3 (upper right), the dispersion of the ethyl acetate concentration in the four beverages is noticeable in *tuba*, *lambanog*, and *tapuy*. For isoamyl alcohol content, there is greater variability in *lambanog* samples followed by *tapuy* samples (Figure 3; bottom left). Meanwhile, there is less dispersion in *basi* and *tuba* but a few outliers are seen for both beverages. The spread of the values of isobutyl alcohol content (Figure 3; bottom right) is again highest in *lambanog* samples. The variability of this VOC is also pronounced in *tapuy* and *basi* while the spread is lowest in *tuba* samples. As expected, the SEM values are also high for the VOCs that exhibit significant variability. As mentioned earlier, increasing sample size can improve SEM values, however, the variability or spread of the values of the four VOCs among samples within a beverage type cannot be predicted. It must be stressed that most of the samples used in this study were sourced commercially and the provenance of many of these samples is difficult to establish. In fact, a study cited the huge variability in the production process among manufacturers of *lambanog* as a major factor that affects the quality of the beverage (Velasco 2013). The same work mentioned that several factors hinder *lambanog*'s global acceptance because product standard is aggravated by problematic issues such as source identification and proper packaging and, more importantly, by the lack of scientific basis on the safety of the product (Velasco 2013). There is also the serious issue of adulteration, a practice prevalent among small peddlers of the drink along the provincial roadside (Velasco 2013). The studies of Ascan *et al.* (2010) and Velasco (2013) point to several factors that limit the important functional areas of *lambanog* manufacturers, which understandably are also applicable to the other traditional spirits under study. The difficulty in ascertaining the exact origin of the samples, as well as the issues mentioned in the foregoing statements, provide a possible explanation why a high dispersion is observed for the VOC levels in the four beverages.

The ANOVA test (p-value) was used to establish any statistically significant differences among the four VOCs' respective mean concentrations in *lambanog*, *tapuy*, *basi*, and *tuba*. Further, Tukey's honest significant difference method was also used to corroborate the ANOVA test and to determine which specific pairs among the four beverages are significantly different in terms of their VOC content. The statistical analysis supports the observed difference in ethanol concentration in the four beverages ($p < 0.05$) and that ethanol levels in *lambanog* are significantly different from those in *tapuy*, *basi,* and *tuba*. Meanwhile, ethyl acetate concentrations are the same ($p > 0.05$) and there are no significant differences in its concentration among the four beverages. The concentrations of isoamyl alcohol in the four beverages are not the same ($p < 0.05$) and only *basi* and *tuba* exhibit significant differences in their isoamyl content. Further, there is sufficient evidence to say that in the four beverages, the concentrations of isobutyl alcohol are not the same ($p < 0.05$). Again, only *basi* and *tuba* show a significant difference in their isobutyl content ($p < 0.05$).

To gain further insight into the nature of the four beverages, a correlation for each pair of selected VOCs is shown in Figure 4. Results of multivariate analysis of the data reveal ethanol is positively correlated to ethyl acetate and isobutyl alcohol. There is a strong correlation between isoamyl alcohol and isobutyl alcohol, which means that samples with high concentrations of isoamyl alcohol would most likely have high concentrations of isobutyl alcohol or *vice versa*. There is no correlation between ethanol and isoamyl alcohol. Ethyl acetate has a weak correlation to isobutyl alcohol. The negative correlation of ethyl acetate to isoamyl alcohol suggests that samples with high concentrations of ethyl acetate would most likely have low concentrations of isoamyl alcohol.

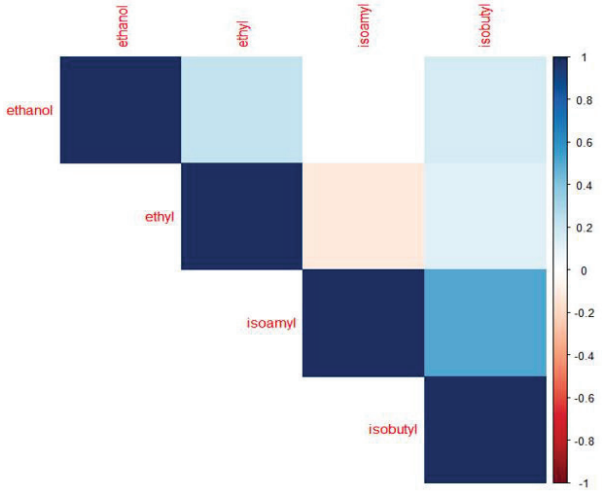

Figure 4. Correlation plot of VOC pairs.

The VOCs quantified in *lambanog*, *tapuy*, *basi*, and *tuba* are among the aroma-active compounds found in most traditional alcoholic beverages in other regions such as China (Chen *et al.* 2013), Korea (Jung *et al.* 2014), and Malaysia (Nur Aimi *et al.* 2013). One of these is ethyl acetate, a potentially important ester that contributes to the fruity character in wines (Gomez-Miguez *et al.* 2007; Sonni *et al.* 2015; Jung *et al.* 2014). Meanwhile, isobutyl and isoamyl alcohol, characterized by their fusel aroma (Gomez-Miguez *et al.* 2007), have also been detected – as in the Japanese traditional wine *sake* – in Korean *makgeoli* (Jung *et al.* 2014) and Thai rice wine *sato* (Amatayakul *et al.* 2012). Apparently, these are major products in the rice wine production using yeast for the fermentation of the rice. These higher alcohols have been reported to lend complexity to the wine, but can cause unpleasant aroma at concentrations higher than 300 mg/L (Amatayakul *et al.* 2012). The VOCs quantified in the *tuba* samples in this study are quite comparable to those in *air nira*, with isoamyl alcohol being among the more important higher alcohols in fermented sap (Nur Aimi *et al.* 2013). Meanwhile, the ethanol content of two *lambanog* samples used in this study corresponds to the usual strength of European spirits that are typically 40% ethanol (Ejim *et al.* 2007). The alcohol strength of *lambanog* samples in this study is also quite similar to that of African *ogogoro*, as reported by Ejim *et al.* (2007).

Preliminary Qualitative Analysis of Selected Samples Using GC-MS

Preliminary studies were embarked on to qualitatively identify other VOCs present in *lambanog*, *tapuy*, *basi*, and *tuba*, especially compounds that may contribute to the sensory properties of the beverages. This was achieved using the GC-MS technique. Only two samples from each beverage type were selected for this purpose. The sample chromatograms are shown in Figure 5 and the corresponding peak tables are reported in Tables VI–IX of the Appendices section.

These chromatograms do not show the complete GCMS profiles of the samples, as MS detection was set to start only after the elution of ethanol at around 4.5 min (solvent cut time). This was done to prevent damage to the detector due to the high concentration of ethanol in these samples. Ethyl acetate was also not detected since it elutes before ethanol.

The higher sensitivity of GC-MS allowed the detection of other VOCs in these alcoholic beverages. Based on the GC-MS chromatograms of the samples, there are certain VOCs that are common in all of these beverages. Apart from ethanol, ethyl acetate, and isoamyl alcohol, compounds such as ethyl lactate and phenethyl alcohol were also found to occur in all four types of alcoholic beverages. Ethyl lactate is commonly found in other

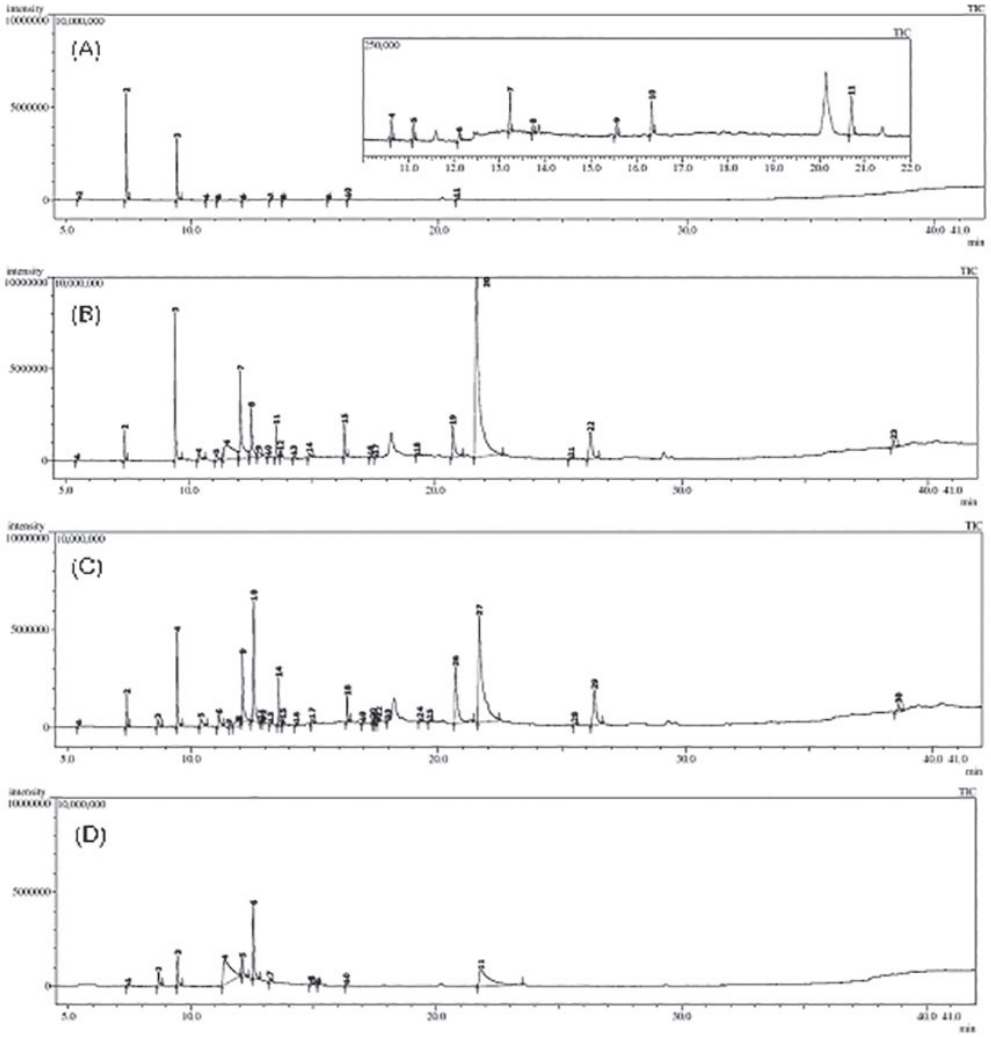

Figure 5. GC-MS chromatograms of *lambanog* (L03; A), *tapuy* (Y03; B), *basi* (B09; C), and *tuba* (T02; D).

alcoholic beverages such as beer and whiskey, which contributes to the fruity flavor of the beverages (Cserhati and Forgacs 2003). Meanwhile, phenethyl alcohol, a common by-product of alcoholic fermentation, is considered to be the most important phenolic higher alcohol in wines and similar beverages (Jackson 2014).

Moreover, acetoin, 2,3-butanediol, butyrolactone, 1,2-cylcopentadione, and glycerol are usual fermentation by-products that are also found in *tapuy*, *basi*, and *tuba* but not in *lambanog*. The absence of these compounds in *lambanog* samples may be attributable to the distillation of *lambanog* that could have eliminated these compounds. Acetic acid is a compound that is absent in *lambanog* but detected in the other three beverages. This accounts for their sour, vinegar-like flavor.

The GC-MS data generated by this study aims to provide additional insight into these traditional beverages, albeit to a limited extent only. It must also be noted that the two samples used for each beverage type cannot be considered "representative" of the respective beverages, hence the recommendation to use in future work more samples whose sources, origins, or manufacturers have been carefully identified.

CONCLUSION

This is the first time that Philippine traditional alcoholic beverages – *lambanog*, *tapuy*, *basi*, and *tuba* – have been subjected to this kind of study. This work specifically quantified analytically-accessible VOCs in the aforementioned beverages. The results from this study (1) demonstrated the applicability of the HS-GC-FID method for the determination of ethanol, ethyl acetate, isoamyl alcohol, and isobutyl alcohol in Philippine

traditional alcoholic beverages; (2) established the range of occurrence of VOCs in each beverage type, as well as the mean values for the VOCs for samples within each beverage type: 35.69% (v/v) ethanol, 329.67 mg/L ethyl acetate, 145.03 mg/L isoamyl alcohol, and 51.86 mg/L isobutyl alcohol for *lambanog*; 10.86% (v/v) ethanol, 212.87 mg/L ethyl acetate, 175.82 mg/L isoamyl alcohol, and 37.98 mg/L isobutyl alcohol for *tapuy*; 11.06% (v/v) ethanol, 114.18 mg/L ethyl acetate, 247.45 mg/L isoamyl alcohol, and 66.54 mg/L isobutyl alcohol for *basi*; and 8.79% (v/v) ethanol, 351.77 mg/L ethyl acetate, 68.74 mg/L isoamyl alcohol, and 16.23 mg/L isobutyl alcohol for *tuba*; (3) showed that the large dispersion of mean values for VOC content observed can be attributed to the difficulty in ascertaining the exact provenance of most of the samples, starting materials used, huge variability in the production process of these beverages, and other existing non-standard practices of makers and retailers; and (4) provided preliminary qualitative information on other families of chemical compounds present in the four traditional beverages, some of which may contribute to their aromatic composition.

This study is a starting point of a scientific inquiry on Philippine traditional alcoholic beverages. The compelling output of this work is the new information generated about the four traditional beverages – *lambanog*, *tapuy*, *basi*, *and tuba*. Making available this information in the scientific literature can certainly open up new interest and appreciation for these beverages by consumers and other stakeholders. This is also an opportunity to provide guidance to consumers as to what substances are actually being ingested during the consumption of these beverages.

Resources permitting, further investigations in the future can be pursued to enable refinement of data on Philippine traditional alcoholic beverages.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

NOTES ON APPENDICES

The complete appendices section of the study is accessible at http://philjournsci.dost.gov.ph

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APPENDICES

Table I. Average VOC levels in lambanog samples.

Sample code	Sample source	Ethanol, % (v/v) (RSD, n=3)	Ethyl acetate, mg/L (RSD, n = 3)	Isoamyl alcohol, mg/L (RSD, n = 3)	Isobutyl alcohol, mg/L (RSD, n = 3)
L01	From commercial manufacturer	37.0 ± 3.0[a] (7.7)	497.1 ± 30.4 (6.0)	ND[c]	151.2 ± 2.8 (2.0)
L02	From commercial manufacturer	40.7 ± 0.4 (0.9)	239.6 ± 8.6 (3.0)	260.9 ± 15.5 (6.0)	ND
L03	From commercial manufacturer	41.1 ± 0.9 (2.1)	637.5 ± 5.8 (0.9)	ND	ND
L04	Lucena, Quezon	32.0 ± 0.8 (3.0)	428.1 ± 33.1 (7.0)	328.1 ± 12.2 (4.0)	93.0[b] ± 4.3 (3.0)
L05	Pitogo Quezon	36.0 ± 1.0 (3.0)	505.4 ± 76.3 (14.0)	516.2 ± 26.5 (5.0)	118.8[b] ± 14.5 (9.0)
L06	Bayog, Laguna	34.6 ± 2.1 (5.8)	ND	ND	ND
L07	San Juan, Batangas	28.4 ± 0.4 (1.4)	ND	ND	ND

[a]Average value ± 95% confidence interval
[b]Based on two replicates
[c]ND – not detected

Table II. Average VOC levels in tapuy samples.

Sample code	Sample source	Ethanol, %(v/v) (RSD, n = 3)	Ethyl acetate, mg/L (RSD, n = 3)	Isoamyl alcohol, mg/L (RSD, n = 3)	Isobutyl alcohol, mg/L (RSD, n = 3)
Y01	La Trinidad, Benguet	12.6 ± 0.1[a] (0.6)	44.1 ± 2.7 (5.7)	235 ± 6.2 (2.5)	49.8[b] ± 0.2 (0.5)
Y02	Cordillera Province	9.88 ± 0.1 (1.1)	264 ± 31.2 (11)	105 ± 8.0 (7.1)	ND[c]
Y03	Baguio, Mt. Province	10.9 ± 0.1 (0.9)	263 ± 9.9 (3.5)	156 ± 0.3 (0.2)	45.2 ± 2.8 (5.8)
Y04	Baguio, Mt. Province	9.01 ± 0.2 (2.1)	498 ± 41.5 (8.0)	107 ± 2.7 (2.3)	26.4 ± 0.1 (0.4)
Y05	Baguio, Mt. Province	8.50 ± 0.1 (1.1)	111 ± 1.6 (1.3)	86.2 ± 1.0 (1.2)	53.7 ± 0.5 (0.8)
Y06	Baguio, Mt. Province	9.39 ± 0.1 (1.2)	38.9 ± 0.7 (1.8)	322 ± 8.0 (2.3)	49.2 ± 0.9 (1.7)
Y07	Baguio, Mt. Province	8.63 ± 0.1 (0.8)	85.7 ± 1.8 (2.0)	203 ± 3.6 (1.7)	36.9 ± 0.8 (2.1)
Y08	Kapangan, Benguet	16.3 ± 0.7 (4.8)	392 ± 11.1 (2.6)	225 ± 43.7 (18)	75.0 ± 8.6 (10.7)
Y09	Unknown	11.3 ± 0.4 (3.5)	139 ± 15.6 (11)	116 ± 3.5 (2.9)	ND
Y10	Bauko, Mt. Province	12.1 ± 1.8 (14)	293 ± 4.1 (1.3)	203 ± 5.5 (2.5)	38.5 ± 1.9 (4.7)

[a]Average value ± 95% confidence interval
[b]Based on two replicates
[c]ND – not detected

Table III. Average VOC levels in basi samples.

Sample code	Sample source	Ethanol, %(v/v) (RSD, n = 3)	Ethyl acetate, mg/L (RSD, n = 3)	Isoamyl alcohol, mg/L (RSD, n = 3)	Isobutyl alcohol, mg/L (RSD, n = 3)
B01	Sta. Maria, Ilocos Sur	10.47 ± 0.2[a] (2.0)	59.26 ± 1.0 (2.0)	438.98 ± 10.1 (2.0)	65.07 ± 2.1 (3.0)
B02	Sta. Maria, Ilocos Sur	13.05 ± 0.1 (0.7)	53.52 ± 2.2 (4.0)	210.45 ± 7.5 (3.0)	47.89 ± 0.7 (1.0)
B03	Sta. Maria, Ilocos Sur	9.55 ± 0.1 (0.9)	49.98 ± 2.5 (5.0)	147.97 ± 12.0 (8.0)	47.93 ± 0.9 (2.0)
B04	San Ildefonso, Ilocos Sur	11.4 ± 0.1 (1.0)	254.9 ± 18.9 (7.0)	236.17 ± 9.5 (4.0)	93.37 ± 4.9 (5.0)
B05	San Ildefonso, Ilocos Sur	11.84 ± 0.3 (2.0)	55.32 ± 3.0 (5.0)	213.86 ± 1.0 (0.5)	88.65 ± 0.9 (0.9)
B06	Pangasinan	10.78 ± 0.02 (0.2)	227.37 ± 5.7 (2.0)	151.83[b] ± 4.0 (2.0)	88.64 ± 4.0 (4.0)
B07	Pangasinan	9.79 ± 0.7 (6.0)	46.02 ± 0.7 (1.5)	233.92 ± 7.5 (3.0)	60.47[b] ± 2.7 (3.0)
B08	Binalonan, Pangasinan	12.12 ± 0.2 (2.0)	436.68 ± 13.1(3.0)	214.14 ± 6.2 (3.0)	65.70 ± 0.8 (1.0)
B09	La Union	8.82 ± 0.3 (3.0)	59.24 ± 1.1 (2.0)	191.30 ± 5.8 (3.0)	35.96±0.6 (2.0)
B10	Laoag, Ilocos Norte	12.74 ± 0.05 (0.4)	27.06 ± 0.5 (2.0)	435.89 ± 59.3 (13)	71.71[b]± 1.1 (1.0)

[a]Average value ± 95% confidence interval
[b]Based on two replicates
[c]ND – not detected

Table IV. Average VOC levels in *tuba* samples.

Sample code	Sample source	Ethanol, %(v/v) (RSD, n = 3)	Ethyl acetate, mg/L (RSD, n = 3)	Isoamyl alcohol, mg/L (RSD, n = 3)	Isobutyl alcohol, mg/L (RSD, n = 3)
T01	Unknown	8.32 ± 0.2[a] (2.0)	135.5 ± 11.2 (8.0)	78.425 ± 3.5 (4.0)	ND[c]
T02	San Jacinto, Masbate	7.04 ± 0.2 (2.0)	117.5 ± 9.4 (8.0)	ND	ND
T03	Naga, Camarines Sur	6.95 ± 0.1 (1.0)	99.79 ± 0.8 (0.7)	ND	ND
T04	Phil. Coconut Authority	8.84 ± 0.1 (1.0)	389.36 ± 5.3 (1.0)	68.83 ± 5.6 (8.0)	ND
T05	Tayabas, Quezon	10.15 ± 0.2 (2.0)	437.35 ± 30.3 (7.0)	60.37 ± 4.5 (7.0)	26.34 ± 0.2 (0.8)
T06	Tayabas, Quezon	8.15 ± 0.2 (2.0)	758.96 ± 57.7 (7.0)	156.35 ± 3.0 (2.0)	36.46 ± 0.2 (0.4)
T07	Tayabas, Quezon	12.96 ± 0.2 (1.1)	558.73 ± 25.6 (4.0)	75.99 ± 3.2 (4.0)	23.95 ± 1.0 (4.0)
T08	Tayabas, Quezon	9.98 ± 0.2 (2.32)	[b]	67.66 ± 1.6 (2.0)	23.95 ± 0.4 (2.0)
T09	San Jose, Antique	6.74 ± 0.2 (2.21)	668.79 ± 11.5 (2.0)	111.01 ± 1.8 (1.5)	35.41 ± 0.3 (0.9)

[a]Average value ± 95% confidence interval
[b]Not quantified due to insufficient sample
[c]ND – not detected

Table V. Standard error of mean concentrations of VOCs.

SEM	*Lambanog*	*Tapuy*	*Basi*	*Tuba*
Ethanol	1.72	0.76	0.45	0.67
Ethyl acetate	92.22	48.95	40.35	91.80
IsoamyI alcohol	76.22	23.69	33.05	16.30
Isobutyl alcohol	25.26	7.49	6.14	5.34

Table VI. VOCs detected in selected *lambanog* samples using DI-GC-MS.

	RT[a]	VOC class	Volatile compound	L03	L05	Odor/taste
1	5.5	Alcohol	Isobutyl alcohol	/[b]	/	Fusel, spirituous (Jung *et al.* 2014)
2	7.4	Alcohol	Isoamyl alcohol	/	/	Unpleasant irritating, upon dilution: fruity, bitter (Jung *et al*. 2014)
3	7.6	Ester	Ethyl caproate	ND[c]	/	Fruity, pineapple-banana, winey (Burdock 2001)
4	9.5	Ester	Ethyl 2-hydroxypropanoate (ethyl lactate)	/	/	Fruity (Chen *et al.* 2013)
5	10.6	Ester	Ethyl caprylate	/	/	Fruity, floral, wine-apricot (Burdock 2001)
6	11.1	Furan	2-furancarboxaldehyde (furfural)	/	/	Sweet, bread-like, caramellic (Flament and Thomas 2002)
7	12.1	Ester	Methyl 2-hydroxyhexanoate	/	ND	No odor description available
8	12.1	Acid	Acetic acid	ND	/	Strong, pungent, vinegar (Burdock 2001)
9	12.6	Alcohol	2,3-butanediol	ND	/	Mildly bittersweet (Jackson 2014)
10	13.2	Ester	Ethyl decanoate	/	ND	Fruity, grape (cognac); oily, brandly-like (Burdock 2001)
11	13.6	Furan	2-Furanmethanol (Furfuryl alcohol)	ND	/	Mild, warm oily, burnt odor / cooked sugar, caramellic (Burdock 2001)
12	13.7	Ester	Diethyl butanedioate (clorius)	/	ND	Faint, pleasant (Burdock 2001)
13	15.6	Ester	Ethyl palmitate	/	ND	Mild, waxy sweet / tasteless, creamy mouthfeel (Burdock 2001)
14	16.3	Alcohol	Benzeneethanol (phenethyl alcohol)	/	/	Rose-like (Jung *et al.* 2014)
15	17.7	Ester	Diethyl malate	ND	/	Fruity, with pleasant herbaceous undertone (Burdock 2001)
16	19.3	Lactone	2-hydroxy-γbutyrolactone	ND	/	No odor description available
17	20.7	Ester	Ethyl-9-hexadecenoate	/	ND	No odor description available
18	26.4	Furan	5-hydroxymethylfurfural	ND	/	Sweet, herbaceous-hay like, caramel (Charalambous 1992)

[a]RT = retention time (min)
[b]"/" = detected
[c]ND = not detected

Table VII. VOCs detected in selected *tapuy* samples using DI-GC-MS.

	RT[a]	VOC class	Volatile compound	Y10	Y03	Odor/taste
1	5.4	Alcohol	Isobutyl alcohol	/[b]	/	Fusel, spirituous (Jung *et al.* 2014)
2	7.4	Alcohol	Isoamyl alcohol	/	/	Unpleasant irritating, upon dilution: fruity, bitter (Jung *et al.* 2014)
3	8.7	Ketone	3-hydroxy-2-butanone (Acetoin)	/	ND[c]	Buttery, bland woody yogurt / fatty creamy (Burdock 2001)
4	9.4	Ester	Ethyl 2-hydroxypropanoate (ethyl lactate)	/	/	Fruity (Chen *et al.* 2013)
5	10.4	Ester	Methyl 2-propenoate (methyl acrylate)	/	/	Sharp acrid (The Dow Chemical Company 2015)
6	11.1	Furan	2-furancarboxaldehyde (furfural)	/	/	Sweet, bread-like, caramellic (Flament and Thomas 2002)
7	11.5	Acid	Acetic acid	/	/	Strong, pungent, vinegar (Burdock 2001)
8	12.3[d]	Alcohol	2,3-butanediol	/	/	Mildly bittersweet (Jackson 2014)
9	12.8	Alcohol	Propylene glycol	/	/	Odorless (Burdock 2001)
10	13.2	Lactone	Dihydro-2(3H)-furanone (γ-butyrolactone)	/	/	Faint, sweet, aromatic, slightly buttery (Burdock 2001)

11	13.5	Alcohol	1-methoxy-2-butanol	/	ND	No odor description available
12	13.6	Furan	2-furanmethanol (furfuryl alcohol)	/	/	Mild, warm oily, burnt odor / cooked sugar & caramellic (Burdock 2001)
13	13.7	Ester	Diethyl butanedioate (clorius)	/	/	Faint, pleasant (Burdock 2001)
14	14.3	Furan	5-methyl-2-furanmethanol	/	/	Weak resinous myrrh woody (Flament and Thomas 2002)
15	14.9	Ketone	1,2-cyclopentanedione	/	/	No odor description available
16	16.3	Alcohol	Benzeneethanol (phenethyl alcohol)	/	/	Rose-like (Jung *et al.* 2014)
17	17.4	Furan	Furyl hydroxymethyl ketone	/	/	Burnt (Sannai *et al.* 1982)
18	17.5	Pyranone	2H-pyran-2,6(3H)-dione (glutaconic anhydride)	/	ND	No odor description available
19	17.6	Furan	2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol)	/	/	Sweet, fruity, strawberry, hot sugar, fruity caramel, burnt pineapple (Burdock 2001)
20	17.8	Acid	Formic acid	/	ND	Pungent, penetrating / acidic, astringent with fruity depth (Burdock 2001)
21	18.0	Lactone	4-hydroxy-5-oxohexanoic acid lactone	/	ND	Wine-like (Perlman 1972); bottle-aged (Jackson 2014)
22	19.3	Lactone	2-hydroxy-γbutyrolactone	/	/	No odor description available
23	19.7	Ether	3-methoxy-pentane	/	ND	No odor description available
24	20.2	Alcohol	Dianhydromannitol	/	ND	No odor description available
25	20.7	Pyranone	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	/	/	Caramel (Flament and Thomas 2002)
26	21.6	Alcohol	1,2,3-propanetriol (glycerol)	/	/	Odorless (Scanes *et al.* 1998)
27	22.0	Amine	N-ethyl-nitroso-ethanamine	/	ND	No odor description available
28	24.3	Ester	Ethyl hydrogen succinate	/	ND	No odor description available
29	25.5	Lactone	(S)-(+)-2',3'-dideoxyribonolactone	/	ND	No odor description available
30	26.3	Furan	5-hydroxymethylfurfural	/	/	Sweet, herbaceous-hay like, caramel (Charalambous 1992)
31	38.6	Phenol	4-hydroxy-benzeneethanol	/	/	Mild beeswax, honey-like (Jackson 2014)

[a] RT = retention time (min)
[b]"/" = detected
[c]ND = not detected
[d]Average of two peaks detected as the same compound

Table VIII. VOCs detected in selected *basi* samples using DI-GC-MS.

	RT[a]	VOC class	Volatile compound	B06	B09	Odor/taste
1	5.4	Alcohol	Isobutyl alcohol	/[b]	/	Fusel, spirituous (Jung *et al.* 2014)
2	7.4	Alcohol	Isoamyl alcohol	/	/	Unpleasant irritating, upon dilution: fruity, bitter (Jung *et al.* 2014)
3	7.8	Ketone	4-ethoxy-2-pentanone	/	ND[c]	No odor description available
4	8.7	Ketone	3-hydroxy-2-butanone (acetoin)	/	/	Buttery, bland woody yogurt / fatty creamy (Burdock 2001)
5	9.4	Ester	Ethyl 2-hydroxypropanoate (ethyl lactate)	/	/	Fruity (Chen *et al.* 2013)
6	10.4	Ester	Methyl 2-propenoate (methyl acrylate)	/	/	Sharp acrid (The Dow Chemical Company 2015)
7	11.1	Furan	2-furancarboxaldehyde (furfural)	/	/	Sweet, bread-like, caramellic (Flament and Thomas 2002)
8	11.5	Ether	cis-5-hydroxy-2-methyl-1,3-dioxane	ND	/	No odor description available
9	11.8[e]	Acid	Acetic acid	/	/	Strong, pungent, vinegar (Burdock 2001)
10	12.3[d]	Alcohol	2,3-butanediol	/	/	Mildly bittersweet (Jackson 2014)

11	12.8	Alcohol	Propylene glycol	/	/	Odorless (Burdock 2001)
12	12.9	Ether	Cis-4-hydroxymethyl-2-methyl-1,3-dioxolane	ND	/	No odor description available
13	13.2	Lactone	Dihydro-2(3H)-furanone (γ-butyrolactone)	/	/	Faint, sweet, aromatic, slightly buttery (Burdock 2001)
14	13.5	Ether	Bis(2-methoxyethyl) ether	/	ND	Mild, ethereal (Cheremisinoff 2003)
15	13.6	Furan	2-furanmethanol (furfuryl alcohol)	/	/	Mild, warm oily, burnt odor / cooked sugar & caramellic (Burdock 2001)
16	13.7	Ester	Diethyl butanedioate (clorius)	/	/	Faint, pleasant (Burdock 2001)
17	14.3	Furan	5-methyl-2-furanmethanol	/	/	Weak resinous myrrh woody (Flament and Thomas 2002)
18	14.9	Ketone	1,2-cyclopentanedione	/	/	No odor description available
19	15.2	Ester	Ethyl-4-hydroxybutanoate	/	ND	Possibly pastry odor (Velasquez *et al.* 2015)
20	16.3	Alcohol	Benzeneethanol (phenethyl alcohol)	/	/	Rose-like (Jung *et al.* 2014)
21	16.9	Pyranone	Maltol	ND	/	Warm, sweet, fruity (Burdock 2001)
22	17.4	Furan	Furyl hydroxymethyl ketone	ND	/	Burnt (Sannai *et al.* 1982)
23	17.5	Pyranone	2H-pyran-2,6(3H)-dione (glutaconic anhydride)	ND	/	No odor description available
24	17.6	Furan	2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol)	/	/	Sweet, fruity, strawberry, hot sugar, fruity caramel, burnt pineapple (Burdock 2001)
25	18.0	Lactone	4-hydroxy-5-oxohexanoic acid lactone	ND	/	Wine-like (Perlman 1972); bottle-aged (Jackson 2014)
26	19.3	Ester	Allyl formate	/	ND	Ethereal, fruity, slightly pungent reminiscent of mustard (Arctander 1969)
27	19.3	Lactone	2-hydroxy-γbutyrolactone	ND	/	No odor description available
28	19.7	Ether	3-methoxy-pentane	ND	/	No odor description available
29	20.7	Pyranone	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	/	/	Caramel (Flament and Thomas 2002)
30	21.7	Alcohol	1,2,3-propanetriol (glycerol)	/	/	Odorless (Scanes *et al.* 1998)
31	25.5	Lactone	(S)-(+)-2',3'-dideoxyribonolactone	ND	/	No odor description available
32	26.3	Furan	5-hydroxymethylfurfural	/	/	Sweet, herbaceous-hay like, caramel (Charalambous 1992)
33	38.6	Phenol	4-hydroxy-benzeneethanol	/	/	Mild beeswax, honey-like (Jackson 2014)

[a] RT = retention time (min)
[b]"/" = detected
[c]ND = not detected
[d]Average of two peaks detected as the same compound
[e]Average of the two samples

Table IX. VOCs detected in selected *tuba* samples using DI-GC-MS.

	RT[a]	VOC class	Volatile compound	T04	T02	Odor/taste
1	7.4	Alcohol	Isoamyl alcohol	/[b]	/	Unpleasant irritating, upon dilution: fruity, bitter (Jung *et al.* 2014)
2	8.7	Ketone	3-hydroxy-2-butanone (acetoin)	/	/	Buttery, bland woody yogurt / fatty creamy (Burdock 2001)
3	9.5	Ester	Ethyl 2-hydroxypropanoate (ethyl lactate)	/	/	Fruity (Chen *et al.* 2013)
4	10.4	Ester	Methyl 2-propenoate (methyl acrylate)	/	ND[c]	Sharp acrid (The Dow Chemical Company 2015)
5	11.1	Furan	2-furancarboxaldehyde (furfural)	/	ND	Sweet, bread-like, caramellic (Flament and Thomas 2002)
6	11.5[e]	Acid	Acetic acid	/	/	Strong, pungent, vinegar (Burdock 2001)
7	12.3[d]	Alcohol	2,3-butanediol	/	/	Mildly bittersweet (Jackson 2014)
8	12.8	Ester	Methyl lactate	/	ND	No odor description available
9	13.2	Lactone	Dihydro-2(3H)-furanone (γ-butyrolactone)	/	/	Faint, sweet, aromatic, slightly buttery (Burdock 2001)
10	13.5	Furan	2-furanmethanol (furfuryl alcohol)	/	ND	Mild, warm oily, burnt odor / cooked sugar & caramellic (Burdock 2001)
11	13.7	Ester	Diethyl butanedioate (clorius)	/	ND	Faint, pleasant (Burdock 2001)
12	14.3	Furan	5-methyl-2-furanmethanol	/	ND	Weak resinous myrrh woody (Flament and Thomas 2002)
13	14.7	Lactone	2(5H)-furanone	/	ND	Sweet, buttery (Maga 1991)
14	14.9	Ketone	1,2-cyclopentanedione	/	/	No odor description available
15	15.2	Ester	Ethyl-4-hydroxybutanoate	/	/	Possibly pastry odor (Velasquez *et al.* 2015)
16	16.3	Alcohol	Benzeneethanol (phenethyl alcohol)	/	/	Rose-like (Jung *et al.* 2014)
17	17.4	Furan	Furyl hydroxymethyl ketone	/	ND	Burnt (Sannai *et al.*1982)
18	17.5	Pyranone	2H-pyran-2,6(3H)-dione (glutaconic anhydride)	/	ND	No odor description available
19	17.6	Furan	2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol)	/	ND	Sweet, fruity, strawberry, hot sugar, fruity caramel, burnt pineapple (Burdock 2001)
20	18.0	Lactone	4-hydroxy-5-oxohexanoic acid lactone	/	ND	Wine-like (Perlman 1972); bottle-aged (Jackson 2014)
21	19.3	Lactone	2-hydroxy-γbutyrolactone	/	ND	No odor description available
22	19.6	Sulfur	1-propene-1-thiol	/	ND	Sulfurous (Verma and Srivastav 2019)
23	19.9	Acid	Sorbic acid	/	ND	[Preservative] (Zoecklein *et al*. 1995)
24	20.7	Pyranone	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	/	ND	Caramel (Flament and Thomas 2002)
25	21.7[e]	Alcohol	1,2,3-propanetriol (glycerol)	/	/	Odorless (Scanes *et al.* 1998)
26	25.5	Lactone	(S)-(+)-2',3'-dideoxyribonolactone	/	ND	No odor description available
27	26.3	Furan	5-hydroxymethylfurfural	/	ND	Sweet, herbaceous-hay like, caramel (Charalambous 1992)
28	29.6	Lactone	Dihydro-4-hydroxy-2(3H)-furanone	/	ND	No odor description available

[a] RT = retention time (min)
[b]"/" = detected
[c]ND = not detected
[d]Average of two peaks detected as the same compound
[e]Average of the two samples

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