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Facile Determination of Antioxidant Activity of Coconut Liquor Using Cyclic Voltammetry

Kyana Renae Cua, Karolyn Clea Villaroman, Eraica Pearl Chan, Danica Allyana Ng, Nathaniel P. Dugos, Allan N. Soriano, Joseph R. Ortenero*

Department of Chemical Engineering, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines
joseph.ortenero@dlsu.edu.ph

In the aspect of sustainable food quality evaluation, reducing the cost and material consumption associated with testing, increasing the efficiency of processing, and reducing the overall environmental footprint are essential considerations. *Lambanog* coconut liquor is a popular distilled spirit in the Philippines, which is sold by producers in diverse quality and compositions. Despite the popularity of coconut vodka, there are few reports on physicochemical properties and antioxidant activity in the literature. This study evaluates the physicochemical and antioxidant activity of this distilled spirit obtained from the Cavite and Laguna provinces in the Philippines. The antioxidant activity determination is carried out using cyclic voltammetry and reported in milligrams equivalent to ascorbic acid (AA). This process is simple and fast, does not require complicated preparation techniques, and no toxic solvents are needed for the evaluation of antioxidant activity. The antioxidant activity evaluated from the anodic curve shows the ascorbic acid equivalents for different brands and found to be 532.0 mg AA, 425.3 mg AA, 381.4 mg AA, 563.2 mg AA, and 284.2 mg AA for Brands A to E. Two brands are acidic, and two are basic. Brand D has the highest range of conductivities (419.9 to 734.4 μS/cm), while Brand E has the lowest (111.4 to 135.4 μS/cm). Future research will explore the chemical constituents present that affect the physicochemical properties of the distilled spirit.

1. Introduction

The Philippines offers a variety of locally produced alcoholic beverages ranging from wine, gin, vodka, and beer. To list a few are the following: *Tuba*, *Basi*, *Lambanog*, *Laksoy*, and *Tapuy*. Among these products, *Lambanog* is highlighted as the subject of this research. Predominantly found in the Southern Province of Quezon, *Lambanog* is a clear, colourless vodka known for its strong, sweet taste. Endo et al. (2014) describe *Lambanog* as a type of palm wine distilled from fermented coconut flower sap and nectar or *Tuba*. *Lambanog* is highly rich in vitamin B_2 or riboflavin, a strong antioxidant that is proven helpful in combating free radicals (Nangia, 2020).
In this study, the physicochemical properties are measured, such as density, pH, and electrolytic conductivity. Measurement of physicochemical properties is important in many applications, such as the design and quality control of food and chemicals, as the final product is influenced by these properties (Igual and Martínez-Monzó, 2022). It is especially true for food products as the physicochemical properties can identify whether it is perishable or requires further treatment to preserve its shelf life. The study of the temperature variation of physicochemical properties can also aid in determining the conditions to which certain food products can be stored and transported to prevent degradation (Martinez and Carballo, 2021). Particularly, density is used to determine the purity and composition of a substance or mixture, and pH is measured to indicate the chemical condition for biological, microbial, and nutritional activity, while electrolytic conductivity is assessed to control food quality.
Antioxidants are compounds that are capable of inhibiting the damaging effects of free radicals. Free radicals are highly unstable atoms or molecules that are capable of damaging the growth and development of cells and tissues due to oxidative stress (Ahmad et al., 2017). These damages often result in various diseases such as cancer, cardiovascular, neurodegenerative, and other chronic diseases (Pham-Huy et al., 2008). Antioxidants

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can break chain reactions because they are stable enough to give up their electrons making a free radical more stable, preventing further cell damage (Romeo et al., 2021).

Over the years, the dangers of oxidative stress have gained popularity, which is why various research has been conducted on various foods that have good antioxidant activity (Montero Fernandez et al., 2018). Examples of such food include fruits, vegetables, teas, herbs and spices, and wines. Fruit wines contain a wide variety of polyphenols, including flavonoids such as anthocyanin, a strong antioxidant known to combat cancer, ageing, infections, and diabetes (De Guzman, 2008). Campanella et al. (2006) measured the antioxidant activity using cyclic voltammetry for herbs extracted using methanolic, acetonic, or aqueous solution. They obtained 726.5 mg equivalent AA from 350 mg extract of Arabica coffee, 442.7 mg equivalent AA from 280 mg extract of Acerola, and 606.6 mg AA from 50 mg Rosemary extract. Antioxidant activity of fruit wines, however, is mostly reported using other techniques such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) (de Souza et al., 2018). Cyclic voltammetry is more attractive because it is fast, easy to use, and does not require complicated preparation of samples. Ortenero et al. (2023) provided a review of the different voltammetric techniques used in the determination of various substances in fruit juices. In addition, much of the research in this field is focused on grape wines, and the extent of research done on other fruit wines is much less in comparison (Čakar et al., 2016).

Lambanog is sold primarily by vendors without proper assessment of the quality, physical, and chemical properties of the liquor. Since most of the producers are small-scale backyard business owners, it has not undergone authoritative assessment. In addition, there is no reported literature on the assessment of antioxidant activity of *Lambanog* using cyclic voltammetry. Due to the simplicity of this process, it could be used as standard method to report the antioxidant activity of the coconut liquor. The result of our study shows antioxidant activity in the range of 284 – 563 mg of ascorbic acid (AA), which is comparable to the value of 243.9 mg AA from 70 mg Açai berry extract and 690.8 mg AA from 250 mg of herb tea extract reported by Campanella et al. (2006).

2. Materials and methodology

The total antioxidant activity of the different brands of *Lambanog* was measured using cyclic voltammetry. The advantages of voltammetry over other methods of antioxidant activity evaluation are easy operation, does not require complicated procedures, and does not involve toxic substances.

2.1 Materials and reagents

Ten (10) L of *Lambanog* were purchased from five Food and Drugs Administration (FDA)-approved brands located in the provinces of Laguna and Batangas. The *Lambanog* was prepared as samples and stored at room temperature for analysis. The reference substance used for the antioxidant activity test was L-ascorbic acid (Sigma-Aldrich ®). KCl was used as the supporting electrolyte. Three-electrode setup consisting of a working electrode (glassy carbon), reference electrode (Ag/AgCl), and counter electrode (platinum wire) was used to perform the CV analysis on the electrochemical cell.

2.2 Experimental procedure

The experiment on the three physicochemical properties (pH, density, and electrolytic conductivity) of *Lambanog* was performed based on a varying temperature of the coconut liquor. Then, cyclic voltammetry was conducted to determine the antioxidant activity of the ascorbic acid and *Lambanog*. For the pH experiment, a calibrated Lutron WA-2015 pH meter was used along with the FLUKE 52 II digital thermometer to accurately monitor the pH and temperature of the sample. The pH meter was calibrated before measuring the pH of each *Lambanog* brand by submerging its probe into a pH 4 or pH 10 buffer solution at room temperature. The probe was washed with deionised water and then completely dried before proceeding with the pH experiment for the 5 brands of *Lambanog*. For the conductivity experiment, the Armfield CEXC fitted with CEM MKII Continuous Stirred Tank Reactor (CSTR) was used along with the accompanying Armfield Software for Batch Reactor in Isothermal Operation.

Before performing conductivity experiments on the *Lambanog* samples, the Armfield CEM MKII CSTR was first calibrated against 0.1 M of KCl standard solution. The standard solution was prepared by weighing 7.24 g of KCl on an analytical balance and diluting it with deionised water in a 1 L volumetric flask. A total of 6 L was prepared and stored in a sealed container to conduct three (3) trials of calibration tests.

For the antioxidant activity experimental set-up, Metrohm Autolab PGSTAT 85649 was connected to a laptop with the pre-installed software (Nova 2.10) where the data was transcribed into. The electrodes (RE, CE, and WE) were immersed in the liquor, and the other end of the electrodes were connected to the potentiostat using an alligator clip. Three-compartment glassware separates the three electrodes with a total liquor volume of 250 mL. KCl was dissolved in the solution to improve the conductivity of the electrolyte. Aqueous standard solutions of ascorbic acid with concentrations of 1.0 mM, 2.0 mM, and 5.0 mM were prepared to serve as references in obtaining the antioxidant activity of the liquor samples. In a separate solution, *Lambanog* from each brand was pipetted into a 250 mL beaker. A 1.0 M KCl solution, as supporting electrolyte, was also added by dissolving 3.73 g of KCl in 50 mL of each *Lambanog* sample. The CV scans for each of the *Lambanog* liquor samples and ascorbic acid standards were measured at a sweep rate of 10 mV/s, applying a potential ranging from -0.3 V to 0.8 V (vs Ag/AgCl electrode). After each cycle, the surface of the glassy carbon working electrode was washed thoroughly. For the standard solutions, the anodic area versus concentration of ascorbic acid calibration curve was generated to determine the ascorbic acid-equivalent antioxidant activity of the liquors. The anodic area of the obtained cyclic voltammogram for the liquors was determined using the online program, SketchAndCalc™.

3. Results and discussion

The physicochemical properties of electrolytic conductivity, pH, and density of Lambanog samples were determined. Cyclic voltammetry was used to measure the antioxidant activity of the different varieties of Lambanog coconut liquor. Ascorbic acid was used as the reference in evaluating the antioxidant activity.

3.1 Physico-chemical properties

The pH reading of the five brands of *Lambanog* against the temperatures of 30 °C to 60 °C is shown in Figure 1. Differences in pH were observed from the pH experiment of the five *Lambanog* brands. Brands D and E were found to be acidic, falling between the pH range of about 4.75 to 5.0, while Brands A, B, and C were basic with a pH range of 7.4 to 8.0. The order of the brands from highest to lowest pH remained constant at all temperatures and was as follows: A > B > C >>> E > D. A one-way ANOVA test with a 5 % significance level confirmed that the differences in pH from the various brands of *Lambanog* were statistically significant from one another, with only Brand A and B being the two brands, whose pH were not significantly different from each other.

There were also several possible causes of the relatively low pH of Brands D and E as compared to the pH range of commercial vodkas. For example, in some cases, citric acid is added to distilled spirits to enhance their flavour, causing a decrease in pH. The age of the *Lambanog* could have also been a factor in the low pH. In a study by Carreon-Alvarez et al. (2016), aged tequilas were found to have lower pH due to the increase in the concentration of organic acids caused by the loss of water during the ageing stage. As *Lambanog* was derived from coconut sap that had been aged for at least 48 h, it could be possible that a similar phenomenon to the study of Carreon-Alvarez et al. (2016) also occurred with the *Lambanog* from Brand D and E, assuming they had both been aged much longer than the other three basic brands.

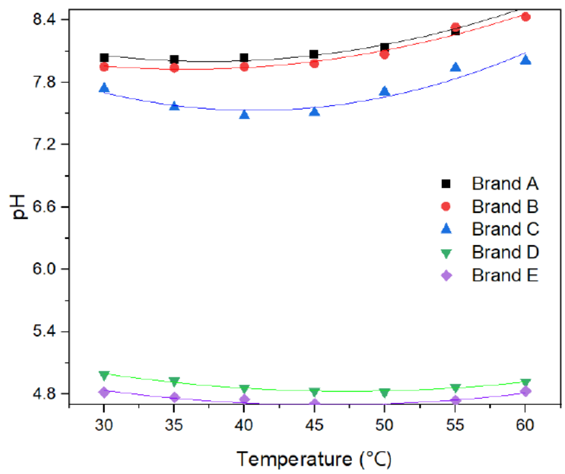

Figure 1: pH of different brands of Lambanog measured at various temperature from 30 °C to 60 °C

Relatively low conductivity values were obtained at temperatures ranging from 30 °C to 60 °C at 5 °C intervals of the different brands indicating that distilled spirits are not good electrolytic conductors. The range of values observed was from 111.37 to 419.88 μS/cm with a mean conductivity of 259.15 ± 112.37 μS/cm at 30 °C and 135.44 to 734.41 μS/cm with a mean conductivity of 411.15 ± 215.58 μS/cm at 60 °C. The order of the brands from highest to lowest set of conductivity values was D >> B > A >> C > E. One of the possible reasons for the higher conductivities of the *Lambanog* brands was the type of water used. In the study by Ejim et al. (2007), the low conductivities of the *Ogogoro* were said to indicate the use of good quality or treated water in liquor production. Similarly, in other studies, the same conclusion was reached wherein those that exhibited lower conductivity suggested the use of treated water, while those with higher conductivity utilized regular tap water (Karapanagioti and Bekatorou, 2014).

By using a hydrometer, the densities of the five *Lambanog* brands were determined. The average density values of the *Lambanog* brands from 30 to 60 °C at 5 °C intervals are 0.960 ± 0.002, 0.957 ± 0.003, 0.953 ± 0.003, 0.951 ± 0.003, 0.948 ± 0.003, 0.945 ± 0.003, 0.942 ± 0.003 g/cm^3. The order of the brands from highest to lowest density was C > A > D > E > B for all temperatures.

3.2 Cyclic voltammogram of ascorbic acid

Cyclic voltammograms for the ascorbic acid standard solutions at different concentrations (1.0, 2.0, and 5.0 mM) are presented in Figure 2. The calculated current density was plotted against the applied potential (vs Ag/AgCl). The current density was obtained by dividing the current by the area of the working electrode having a measured diameter of 3.0 mm.

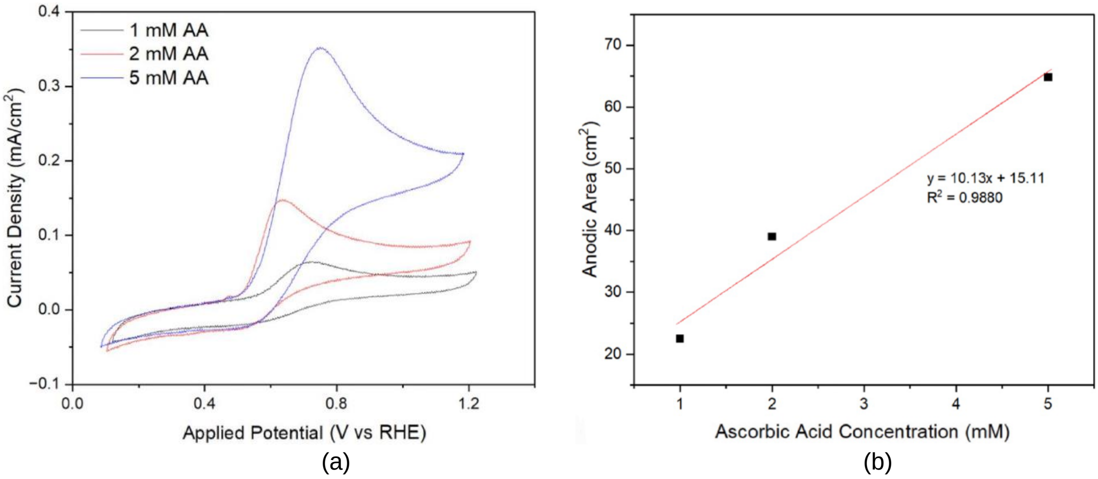

Figure 2: (a) Ascorbic acid voltammogram for three concentrations (1.0 mM, 2.0 mM, and 5.0 mM) using 1.0 M KCl supporting electrolyte, (b) Calibration curve for the anodic area and ascorbic acid concentration

The obtained cyclic voltammograms essentially describe the oxidation of the analyte, ascorbic acid, into dehydroascorbic acid at the surface of the working electrode. Initially, the applied potential was not sufficient to oxidise the ascorbic acid. The current reading was very close to zero. As the potential continued to increase, the analyte began to oxidise and diffuse into the surface of the working electrode at an increasing rate, causing a subsequent exponential increase in the anodic current until an anodic peak was achieved. After the peak, the current decreases due to limitation by the mass transport of the ascorbic acid in the bulk solution onto the diffuse double layer (DDL) of the electrode. As diffusion continued to occur, the DDL grew, slowing down the mass transport of ascorbic acid and causing the anodic current to decrease. When cycled backwards, the presence of a cathodic peak would have indicated the reduction of dehydroascorbic acid to ascorbic acid. However, as there was no observed reduction peak after the switching potential was applied, this study confirmed the reported data from the literature that the electrochemical oxidation reaction of ascorbic acid is an irreversible process (Pisoschi et al., 2011).

Other than anodic current versus concentration, another important relationship to establish, especially for the antioxidant activity determination of *Lambanog* using CV, was that of the anodic area of the voltammogram and the concentration of ascorbic acid. From the obtained voltammograms in Figure 2a, the area of the region bounded by a horizontal baseline at a current density of 0 mA/cm^2 and the voltammogram curve was measured using the online program, SketchAndCalc™. The program adopts the shoelace algorithm, otherwise known as Gauss' area formula, in determining the area under the curve. Plotting the integral anodic area of the voltammogram curve against the concentration of the ascorbic acid gave a calibration curve for measuring antioxidant activity, shown in Figure 2b, which would be referred to in the latter portion of the discussion to measure the antioxidant activity of *Lambanog* in terms of concentration of ascorbic acid.

3.3 Cyclic voltammogram of Lambanog samples

In the same way as ascorbic acid, the *Lambanog* samples were subjected to the CV test, and their corresponding cyclic voltammograms were obtained as collated in Figure 3.

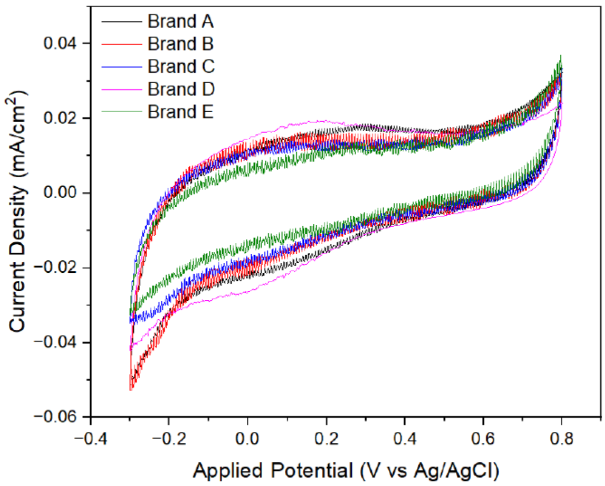

Figure 3: Cyclic voltammograms of the five Lambanog brands vs Ag/AgCl electrode

Visually, it can be observed that the different brands have approximately similar anodic area. To verify this, the area under the anodic current wave with the baseline at 0.0 mA/cm^2 was obtained using the same online program used for the ascorbic acid voltammograms. The order of the brands from highest to lowest anodic area can be ranked as follows: D > A > B > C > E. Using the calibration curve in Figure 2b, the areas of the different brands were plotted to obtain their corresponding concentration in terms of ascorbic acid is given in Table 1. Overall, the amount of equivalent ascorbic acid present in *Lambanog* was found to fall within the range of 284.2 to 532.0 mg or an average of 437.2 ± 101.5 mg.
The study conducted by Roselló et al. (2020) on 19 beer brands of different varieties in Spain showed similar cyclic voltammograms as that of the ones produced from *Lambanog.* Both figures showed similar leaflike-shaped cyclic voltammograms, and there were no noticeable peaks observed from both the *Lambanog* and the beer samples. However, they have not reported the activity of the beers in mg of AA, so a numerical comparison of value is not possible. In the study conducted by Campanella et al. (2006) for the antioxidant activity of herbs and teas, they obtained 243.9 mg AA from 70 mg of Açai berry extract and 690.8 mg AA from 250 mg of herb tea extract.

Table 1: Anodic area and corresponding ascorbic acid concentrations

Lambanog Brand	Area (cm^2)	Equivalent Ascorbic Acid (mg)
Brand A	45.70	532.0
Brand B	39.56	425.3
Brand C	37.04	381.4
Brand D	47.49	563.2
Brand E	31.45	284.2

Based on the results, the antioxidant activity of *Lambanog* showed a positive and strong correlation with conductivity and alcohol content (> 0.7) but showed poor correlation with pH and density (< 0.5). Conductivity was found to have a positive and moderate correlation with alcohol content (0.5 to 0.7), a poor correlation with density, and a negative and poor correlation with pH. Lastly, pH was found to have a positive and moderate correlation with alcohol content, but a negative and poor correlation with density.
The differences in equivalent ascorbic acid antioxidant activity show the differences in quality of the different brands. These differences could be due to differences in sources of coconut sap, processing techniques, and additives. Further studies are necessary to investigate the various polyphenols, i.e. flavonoids, that could contribute to the antioxidant activity of the fruit wine.

4. Conclusions

A well-known alcoholic beverage made in the Philippines, *Lambanog,* a spirit distilled from fermented inflorescence sap, was investigated for physicochemical properties and antioxidant activity. The physical properties of the Lambanog brands show the pH of the three brands as slightly acidic, and two are weakly alkaline. The electrolytic conductivity ranges from 111.37 to 419.88 μS/cm with a mean conductivity of 259.15

± 112.37 μS/cm. The antioxidant activities of the *Lambanog* brands A to E are 532.0, 425.3, 381.4, 563.2, and 284.2 mg ascorbic acid equivalents per 50 mL of the liquor. However, their cyclic voltammograms do not display any distinct peaks. It is recommended that future studies explore other antioxidant activity determination methods, particularly spectrophotometric methods, and compare their findings with that of CV. This can serve as a way to validate the accuracy of CV and expand the knowledge and information about the antioxidant properties of *Lambanog*. Given the versatility and flexibility of CV, there is a great advantage of using this method for a wide variety of samples, including other beverages, food extracts, and even biological materials. Experimenting with other beverages, particularly local beverages, shows great potential for future research, especially given how antioxidant information on these types of beverages is currently still quite limited.

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