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Asian Journal of Plant Science and Research, 2022, 12(6)

ISSN : 2249-7412
CODEN (USA): AJPSKY

Development of Red Fruit Wine Utilizing Bamboo Leaves and Indigenous Fruits

Mariluz SP Dionglay[1], Rowena E Ramos[1*], Aida B Lapis[2], Erlina I Dizon[3], and Bernard O Bawing[4]

[1]*DOST-Forest Products Research and Development Institute, Los Banos, Laguna, Philippines*

[2]*DENR-Ecosystems Research and Development Bureau, Los Banos, Laguna, Philippines*

[3]*UPLB-College of Agriculture and Food Science (CAFS)–UPLB, Los Banos, Laguna, Philippines*

[4]*CS First Agri-Industrial Development Incorporated, Bayambang, Pangasinan, Philippines*

***Corresponding author:** Rowena E Ramos, DENR-Ecosystems Research and Development Bureau, Los Banos, Laguna, Philippines, E-mail: rowena.ramos@fprdi.dost.gov.ph

Received date: May 2, 2022, Manuscript No. AJPSKY-22-13492; **Editor Assigned date:** May 3, 2022, PreQC No. AJPSKY-22-13492 (PQ); **Reviewed date:** May 18, 2022, QC No. AJPSKY-22-13492; **Revised date:** May 24, 2022, Manuscript No. AJPSKY-22-13492 (R); **Published date:** June 1, 2022, DOI: 10.36648/2249-7412.12.6.218

Citation: Dionglay MSP, Ramos RE, Lapis AB, Dizon EI, Bawing BO (2022) Development of Red Fruit Wine Utilizing Bamboo Leaves and Indigenous Fruits. J Rare Disord Diagn Ther Vol.12 No.6: 218

ABSTRACT

This study was carried out to explore the potential of the leaves of 'kauayan-tinik' (Bambusa spinosa Roxb.) species of bamboo as fortifier in the production of red fruit wine using local bignay (Antidesma bunius (L.) Spreng) and lipote (Syzygium polycephaloides (C.B. Rob.) Merr.) fruits. The phytochemical components of pure bamboo ('kauayan-tinik') leaf extracts were qualitatively determined. The leaves were found to contain primary and secondary metabolites such as tannins, terpenoids, flavonoids, cardiac glycosides, carbohydrates, and reducing sugars.

Production of lipote and bignay wines adopted the standard method of red wine processing using Saccharomyces ellipsoideus as the starter, modified by infusion with fresh bamboo leaves during fermentation and carbonation of the finished products. Both lipote wines and bignay wines showed high Phenolic Content (PC) and free-radical scavenging activity. The use of more leaves resulted in higher PC and DPPH with bignay wines showing higher values than lipote wines for PC.

All treatments with bamboo leaves gave higher inhibition activity compared to the control antioxidant, butylatedhydroxy-toluene or BHT (56%), with lipote wines (88%) exhibiting higher values than bignay wines (76%). Sensory analysis showed that among the samples, that red fruit wine variants with bamboo leaves were generally acceptable to the panelists (N=15).

Keywords: Phytochemicals; Red fruit wines (lipote and bignay); Phenolic content; DPPH radical scavenging; Sensory test

Introduction

The Philippine bamboo industry has been supported by the government *via* the promulgation of the Philippine Bamboo Industry Development Act of 2017 to make it competitive to provide opportunities for local employment and to establish bamboo-based enterprises locally and abroad. Unlikely to be called "poor man's timber" such as then, bamboo is now being used in the Philippines for creating furniture and fixtures for schools, as well as for other construction requirements of government facilities [1].

Technological innovations have introduced an array of diverse products commercially available in the market today. Global competitiveness is the key element in moving technically equipped professionals to use their ingenuity and imagination to suit customer's specific needs. New products should be economically viable to provide profit margins and survival in its target markets. Thus, the need for strong investment in research and development should be realized. Technology is of little value if we are not able to utilize it to produce a superior price-valued product. Also, the importance of integrating technological plans such as process standardization and monitoring should be paralleled with business and economics. These two, technology and business, are integral part of product commercialization.

The Philippines is blessed with tropical climate suitable for a vast flora of exotic fruits and grasses. Majority of the locally cultivated fruits have been tested in the laboratories for the production of various types of products. These include juices, powder, puree, alcoholic beverages among others. On the grass species however, particularly bamboo, development of food products for commercialization is limited. Creation of new or innovative product lines to suit the changing lifestyle of present day consumers will make different fruits and grasses in the market available whole year round.

Wine production in the country is confined to limited niche markets from local fruits as mango wine, casoy wine, strawberry wine, bignay wine, lipote wine, lituko wine, to name a few. Often times the source of local fruit wines are close to where raw materials are found and located. The production is confined only at home based level and hardly reached the commercial scale. The demand for local wines even domestically is also driven by the current quest for organically produced ingredients.

The production of beverages with herbal infusions, bamboo beer, and other beverages for commercial use was well documented in some studies and prior arts. For instance, as the leading exporter and processing of bamboo products, China in recent years promoted the beer that claims to have nutritive value and with multiple health benefits and typical delicate fragrance of bamboos. The production of bamboo wine was also reported which uses fresh bamboo juice through pulverizing bamboo and adding amylases for saccharification of the material prior to brewing and fermentation. Another citation of bamboo wine production was that bamboo liquid is obtained by collecting from the growing bamboo plants, and also by soaking parts of bamboo and then combining it with wine to make the final wine formulation [2-6].

Kauayan-tinik (Bambusa spinosa Roxb.) is an arborescent bamboo, occasionally shrubby or scrambling. It is also known as spiny bamboo or thorny bamboo, and is a species of bamboo occurring in Tropical Asia (Poaceae). Rhizomes are short-necked. Stems are 10 meters to 25 meters high, 8 cm to 15 cm in diameter, the basal parts surrounded by stiff, branched, interlaced, spiny branches. It rarely produces flower and its panicles are large. Spikelets are slender, compressed and 2 cm to 3 cm long. Leaves are flat, thin, smooth-edge, alternate arrangement, parallel pattern of main veins, no hairs, 10 cm to 20 cm long, 1 cm to 2 cm wide. The fresh kauayan-tinik bamboo leaves for this study werecourtesy of the CS First, Bayambang, Pangasinan.

Different pharmacological activities and biological effects of bamboo grass were reported, such as anti-tumor activity, anti-free radical, anti-aging, and the prevention of cardiovascular diseases [7]. Phytochemicals (e.g. flavonoids) present in plants and the food we take in have yielded global interest about concerning human health effects, for example, as antioxidants, and prevention of diabetes and tumor (Seki et. al., 2008).

This study promotes the utilization of commonly unused parts of the bamboo (e.g. leaves) by developing these into higher-valued products, as well as maximize its use as additive extract component in bamboo wine to enhance the beverage's flavor and health benefit. Previous studies of the health benefit of bamboo leaves include phytochemicals, antimicrobial, and antioxidant properties of various bamboo leaf extracts which can be used for product development. Product development on bamboo provides a livelihood platform in agricultural rural areas and will generate sustainable livelihoods. Kauayan-tinik (Bambusa spinosa Roxb.) is an arborescent bamboo that is occasionally shrubby or scrambling. Different pharmacological activities and biological effects of bamboo grass were reported, such as anti-tumor activity, anti-free radical, anti-aging, and the prevention of cardiovascular diseases [7]. Phytochemicals (e.g. flavonoids) present in plants and the food we eat have sparked interest with regards to human health benefits, for instance, as antioxidants and prevention of diabetes and tumor (Seki et. al., 2008).

Java plum (Syzygium polycephaloides (C.B. Rob.) Merr.), or lipote, which is indigenous to the Philippines, usually occurs in clusters, small, round and about 20 mm in diameter. 3Lipote is under the fleshy-fruited sub-family Myrtoideae which are often described by their bright anthocyanin colors such as orange, red, purple and black. The fruits were said to have dry but of pleasant acid flavor and is a good source of protein and rich in vitamin C [8].

Bignay (Antidesma bunius (L.) Spreng) on the other hand belongs to the family Euphorbiaceae and is known by different names in the Philippines such as bignay-kalabaw (Tagalog), bugnay (Ilokano, Ibanag and Bisaya), buglay

(Bontok and Ifugao); as well as in other countries such as buni or buneh (Malaysia), wooni (Indonesia) and ma mao luang (Thailand [8]. Among its English names are Chinese laurel, currant tree, nigger's cord, and salamander tree (Morton, 1987). Bignay fruit is thin-skinned, spherical to ovoid, dark red to black when ripe, small (8 mm-10 mm long) and has a sour to sub-acid unique flavor. Bignay fruit contains provitamin A, vitamin C, vitamin E, vitamin B1, iron, minerals, fiber, potassium and phosphorus. They are also made into excellent jam or attractive red jelly, refresh-ing drink and yield an excellent wine.

This project also supports the national programs of DOST on bamboo science and technology by developing higher-valued natural products which may assist the communities through livelihood programs of local government units in the future. This study aimed to show the potential nutraceutical use of kauayan-tinik leaves as fortifier of fruit wine.

Materials and Methods

Procurement of raw materials and source of yeast culture

Fresh and matured lipote fruits were procured from Quezon province while bignay fruits were purchased from Los Banos, Laguna. The fresh kauayan-tinik bamboo leaves were courtesy of the CS First, agri-industrial, Bayambang, Pangasinan. Packaging materials were procured from various glass wares and chemical suppliers in Metro Manila and Laguna areas. The wine yeast (Saccharomyces ellipsoideus) was obtained from the food microbiology laboratory, institute of food science and technology, college of agriculture and food science, University of the Philippines Los Banos, Laguna.

Wine processing

Lipote and bignay fruits were processed into carbonated red wine. Pulp fermentation was implemented for red wine making. The lipote and bignay wines were prepared following the optimized method of the institute of food science and technology (UPLB) for red wine fermentation. The general flow diagram of red wine production was shown in Figure 1.

Figure 1: General flow of red wine production.

The processing of red fruit wines, basically followed the procedure for red wine fermentation. The dilution for bignay fruits was 1:3 (1 part bignay fruit: 3 parts water). After adjustment of sugar content to 20°C, the mixture was also divided equally to three parts: C (control), T1 (with 1 g bamboo leaves per 100 ml must solution, 1% bamboo leaves), and T2 (bignay with 2 g bamboo leaves per 100 ml must solution, 2%). Same set-up was made for lipote fruits but witha dilution of 1:2 (1 part lipote fruits: 2 parts water)

To free the mixture from spoilage microorganisms, 5 mL of 10% sodium metabisulfite solution was added per gallon. For each treatment, 10% of the must was separated in Erlenmeyer flasks for starter preparation, prior to addition of sodium metabisulfite solution.

Starter preparation

As mentioned above, 10% of the mixture was placed in a separate Erlenmeyer flask and covered with cotton fitted with aluminum foil for starter preparation. These were then pasteurized in a boiling water bath for 25 minutes and cooled down to around 40°C-45°C prior to inoculation/addition of starter yeast (Saccharomyces ellipsoideus). The starter was allowed to ferment for 16 hours-18 hours and then added to the prepared "must" in gallon jars.

Alcohol fermentation and filtration

After addition of yeast starter, the "musts" were fermented for 2 days-3 days using only aluminum foil or cotton plug for covering the fermentation jars. The "musts" were then filtered using plastic screen to separate the skin and seeds of the fruit from the fermenting extract. The resulting extracts were poured back to their respective jars and immediatelycovered with an airlock for 3 weeks-4 weeks anaerobic fermentation.

After fermentation, as evidenced by the absence of bubbling in the airlock, the wine was filtered and transferred to a clean jar. Sodium metabisulfite solution (10%) was added at the rate of 5 mL per gallon of raw wine to totally stop yeast activity. The wine from various treatments was allowed to stand for a month for natural sedimentation/clarification.

Physico-chemical analyses

The pH and Total Soluble Solids (TSS) of the diluted fruit pulps and wine after fermentation were determined using a Milwaukee pH pen/meter (2019) and hand refractometer (Atago brand 2012), respectively. In addition, the alcohol content (%) of the wine after fermentation was determined by distillation process and alcohol meter (UPLB college of agriculture and food science) (Figure 1).

pH: The pH meter was calibrated with pH 4.0 buffer and countercheck with pH 7.0 buffer. Approximately 50 mL of the sample was poured into the beaker and then the pH pen electrode was dipped. The pH reading was recorded.

Total Soluble Solids (TSS): An appropriate amount of sample was placed on the prism of the refractometer. The reading was taken directly on the line formed by the light and dark areas and the TSS value was expressed as °Brix.

Alcohol content (%): One hundred (100) mL sample and 50 ml distilled water were measured and placed in 350 mL capacity Erlenmeyer flask. Then, the mixture was distilled and approximately 75 ml distillate was collected. The distillate in graduated cylinder was filled up to 100 mL with distilled water and mixed well. Cover with aluminum foil and refrigerate for 15 minutes-20 minutes. The alcohol meter was gently placed onto the liquid and allowed it to float freely on the sample. The reading was taken directly on the meter.

Total phenolic content and free-radical scavenging activity by DPPH assay

Total phenolic content: The total phenolic content of lipote and bignay wine samples was determined by Folin-Ciocalteu method following the procedure of [9]. The sample was diluted to 25 mL with ethyl alcohol and then to 0.5 mL of the samples, 2.5 mL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich) and 2.0mL Na_2CO_3 were added then mixed with a vortex mixer. Standard samples were placed in 45°C water bath for 15 mins and cooled down. The absorbance readings were measured at 765 nm (Shimadzu UV-1601 spectrophotometer) with water plus reagent as blank samples. Total phenolic content was computed in a standard curve with gallic acid as reference phenol. The results were expressed as gallic acid equivalents (GAE mL-1).

Antioxidant activity (free-radical scavenging activity): The antioxidant activity of polyphenols was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) according to the method of Ribeiro et al. (2008) with some modifications, using synthetic antioxidant Butylatedhydroxy-Toluene (BHT) as reference antioxidant with 1 mg/mL concentration.

Five mL (5 mL) of 0.1 mM DPPH were pipetted in test tubes and added with 0.5 mL of the standards and samples. Samples were diluted to 25 mL. The mixture was vortexed and allowed to stand for 20 minutes. Ethanol (0.5 ml) served as control. The absorbance of the solution was read at 517 nm against reagent blank (Shimadzu UV-1601 spectrophotometer). The percentage of inhibition was expressed using the following equation:

% Inhibition=((A_control- A_Sample)/A_control) × 100

Qualitative metabolites and phytochemical contents of 'Kauayan-Tinik'

Phytochemical screening: Chemical test was done on the ethanolic bamboo leaf extracts for the qualitative determination of plant metabolites and phytochemical constituents using the methods described by Harborne; Tease and Evans; Sofowora [10-12]. The ethanolic bamboo leaf extracts were extracted *via* rotaevaporation at 45°C (Cole Parmer N-1001). The methods performed, reagents used, and positive reactions was summarized as Table 1.

Phytochemical constituent	Reagent used/test performed	Positive Indication/presence of compound
Alkaloids	Mayer's reagent	White or cream precipitates
26	Dragendorff's reagent	Reddish brown precipitates
Saponins	Frothing test	Creamy mass of bubbles
Tannins	Ferric chloride (0.1%)	Brown-green or blue-black precipitates
Flavonoids	Mg ribbons/hydrochloric acid	Pink, scarlet, crimson red, green or blue-black appearance
Steroids	Acetic anhydride/sulfuric acid	Color change from violet to blue or green
Terpenoids	Chloroform/sulfuric acid	Reddish brown interface
Cardiac glycosides	Keller-Killiani test	Brown ring at the interface, violet and green rings may also appear below and above the interface, respectively
Carbohydrates	Molisch's test	Purple ring at the junction
Reducing sugar	Fehling's test	Brick red precipitates

Table 1: Summary of phytochemical screening conducted in kawayan-tinik ethanolic extract.

Sensory evaluation: Sensory evaluation was conducted to determine the sensory attributes and acceptability of carbonated lipote and bignay wines added with different concentrations of bamboo leaves (1% and 2%) and adjusted to 10°Brix. The products were subjected to sensory test using modified scale with 7 points (e.g. 7-point Likert scale) [13]. The samples were compared with the control (no added bamboo leaves).

The wine samples were aged for a month prior to sensory evaluation by 15 member panel composed of people familiar with drinking alcoholic drinks. The products were evaluated for color, mouthfeel (alcohol), sweetness, aroma, flavor (grassy), off-flavor and general acceptability. Wines were served cold, together with a glass of drinking water, in coded shot glasses to the panelist. The sensory test was conducted at CS First Green, Agri-Industrial Bayambang, Pangasinan, on June 26, 2019 (Table 1).

Results and Discussion

Physico-chemical properties of Lipote and Bignay wines

The physico-chemical properties of Lipote and Bignay wines (pH, TSS and alcohol content) as influence by fermentation and addition of bamboo leaves (1%-2%) were evaluated. Both the pulp of Lipote and Bignay fruits were diluted with water at the rate of 1:3 (Pulp:Water) while their TSS was adjusted to 20°Brix prior to alcohol fermentation to produce the right alcohol content for wines.

Ph: The total titratable acids and pH, in general, may be related in terms of the acid present in the substance (Amerine and Ough, 1974).

Most pathogenic microorganisms are killed at lower pH which in turn aids in the preservation of wine. Some pigment also such as anthocyanin is in its oxidized form giving it a vivid red color in acidic conditions. Table 2 shows the pH, TSS (°Brix) and alcohol content of the different dilutions lipote and bignay, respectively (Table 2).

Since the study was conducted with a year time frame, the wine processing developed by UPLB CAFS was adopted and average values per indicated parameter (N=2) were made for the fruit collection of the batch available only for that season. In this study, the pHs of control wines both decreased after fermentation; pH 2.90-2.70 for lipote wine and pH 3.2-pH 3.15 for bignay wine. This may be associated with the conversion of sugars; present in fruit must, into organic acids by the yeast starter. An increase in the concentration of hydronium ion in the solution is released as reflected by the decrease in pH and acidic conditions.

Parameter	pH	Total soluble solids (°Brix)*	Alcohol content (%)
Lipote wine			
1:3 dilution	2.9	3	-
(1 part fruit:3 parts water)			
After fermentation			
T1–Control (LC)	2.7	17	2.00+0.0
T2–1% LBL	3	7	10.80+0.3
T3–2% LBL	3	9	9.00+0.0
Bignay wine			
1:3 dilution	3.2	2.4	-
(1 part fruit:3 parts water)			
After fermentation			
T1–Control (BC)	3.15	7.2	10.0+0.0
T2–1% BBL	3.6	6	10.2+0.6
T3–2% BBL	3.6	6	11.0+0.0

Table 2: Changes in the physico-chemical properties (pH, TSS, and alcohol content) of lipote and bignay wines as affected by fermentation and addition of bamboo leaves. *TSS adjusted to 20°Brix prior to yeast fermentation; LBL: Lipote wine infused with bamboo leaves (w/v); BBL: Bignay wine infused with bamboo leaves (w/v).

However, both wines fortified with bamboo leaves showed increased in pH values after fermentation. The lipote wines with 1% and 2% bamboo leaves both obtained pH 3.0 while bignay wines have 3.60.

It is important to note that the pH of wine also depends on the variety and degree of ripeness of the fruits. The available lipote and bignay fruits at the time the wines were processed may not have been fully ripe hence have high acidity thus, yielding a low pH value.

Total Soluble Solids (TSS) and alcohol content: The TSS value reflects the sugar concentration in addition to other solids such as minerals that may refract light present in the solution. The TSS value can be used as an approximate for calculating the alcohol yield in the wine product [14]. On the other hand, alcohol concentration is the percentage of distillable alcohol (eg. ethanol) in the wine which is dependent on the sugar content of the fruit must. It contributes to the astringency, aroma and aftertaste of the wine product.

A variety of Saccharomyces species is a well-known in alcoholic beverage making (e.g. beer and wine) having a main purpose to metabolize sugars to alcohol, carbon dioxide and other metabolites without development of undesired food constituents and flavors [15].

Results revealed that the TSS of lipote and bignay pulps after dilution with water (1 part pulp:3 parts water) was 3.0°Brix and 2.4°Brix, respectively. The fruit "musts" were both adjusted to 20°Brix through sugar addition before fermentation.

As shown in Table 1, the final TSS of both lipote and bignay wines decreased. The TSS of lipote after fermentation was 17.0°Brix for the control indicating very slow fermentation to none at all while the samples fortified with bamboo leaves ranged from 70°Brix-9.0°Brix.

In the case of bignay wines, no considerable differences were observed in all samples, a steady decreased in TSS was observed giving low values of 6.0°Brix-7.20°Brix at the end of 3 weeks-4 weeks of fermentation. The decrease in TSS can be explained by the activity of yeast, capable of converting sugars to carbon dioxide and ethanol under anaerobic condition. As the TSS decreased, corresponding increase in alcohol content values was obtained. The final alcohol content of bamboo leaf-fortified samples ranged from 9.0%-10.8% for lipote and 10.2%-1.0% for bignay wines.

Phenolic Content and Antioxidant Activity

The phenolic antioxidants in red wines have been proposed as an explanation for the lower death rate from Coronary Heart Disease (CHD) in France referred to as "The French Paradox." Despite high fat intake, mortality from CHD is lower in some regions of France than in the other developed countries due to regular wine consumption [16]. Phenolic compounds in wines play an antioxidant role in both biological and food systems. They have many favorable effects on human health, such as inhibition of oxidation of low-density lipoprotein cholesterol, and inhibition of platelet aggregation thereby potential aid in decreasing heart disease risks [17].

Antioxidants are synthesized or natural substances that may prevent or delay some types of cell damage by donating electrons or hydrogen atoms to reactive free radicals [17]. Antioxidants are found in many foods, including fruits, vegetables and wine. A lot of wine components, including several hundred different phenols, possess strong Antioxidant Activity (AOA) and thus are common research topic. The phenolic content of wine refers to large group of chemical compounds that affect its taste and color. These compounds include phenolic acids, stilbenoids, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins) and flavanol polymers (proanthocyanidins) [18].

The phenolic content and antioxidant activity of lipote and bignay wines fortified with 1% and 2% bamboo leaves and BHT, a known synthetic antioxidant are given in Table 3. Due to limited amounts of the wines produced, control samples were not analyzed.

Sample	Average phenolic content (mg GAE/L wine)**	DPPH (% Inhibition) ***
Lipote wine without fresh bamboo leaves	-	-
Lipote wine with 1% fresh bamboo leaves	272.26	85.81
Lipote wine with 2% fresh bamboo leaves	399.85	89.34
Bignay wine without fresh bamboo leaves	-	-
Bignay wine with 1% fresh bamboo leaves	411.57	78.65
Bignay wine with 2% fresh bamboo leaves	397.87	73.66
BHT* (1 mg/mL)	-	56.14

Table 3: The phenolic content and antioxidant activity of lipote and bignay wines fortified with 1% and 2% bamboo leaves. *BHT: Butylatedhydroxy-Toluene (a synthetic antioxidant); **: Gallic acid equivalent per mL sample;***DPPH: Radical scavenging ability using 2,2-diphenyl-1-picrylhydrazyl (DPPH); -:No analysis due to limited samples; ****: Average of 3 replicate values.

The PC of lipote wine were 272.26 mg-399.85 mg GAE/L for the treatment with 1% and 2% bamboo leaves, respectively. The recorded higher mean values of PC at 2% were contributed by the bamboo leaves.

The same trend was observed for the free radical scavenging efficacy by DPPH, with % inhibition higher for treatment with higher level of bamboo leaves which ranged from 85.81%-89.34%. Moreover, the lipote wine samples showed substantially higher antioxidant activity than the standard BHT (56.14%).

The PC of bignay wines was higher (411.57 mgGAE/L) than the lipote wine fortified with 2% bamboo leaves (399.85 mgGAE/L) but no considerable difference for samples with 1% bamboo leaves. In the case of radical scavenging activity by DPPH, the lipote wines obtained higher values (85.81%-89.34% inhibition) than bignay wines (73.66%-78.65% inhibition). Moreover, as compared to the standard BHT (56.14% inhibition), both lipote and bignay wines fortified with bamboo leaves have higher antioxidant properties.

The results mean that the red wines produced from these fruits fortified with bamboo have phenolic and antioxidant contents, although there might not be sufficient evidence that an increase of bamboo leaves from 1% to 2% will provide a significant increase in free-radical scavenging abilities.

Qualitative Metabolites and Phytochemicals Contents of ‘Kauayan Tinik’

Plants are capable of synthesizing a wide variety of chemical compounds that perform important biological functions which could be used for food, medicinal, and pharmacological purposes. Extraction and characterization of several active phytocompounds from plants have given rise to some high activity profile drugs because of the reports that these show antioxidant or free radical scavenging properties. These phytochemicals are divided into primary and secondary metabolites. Primary metabolites are essential to all plants such as sugars, fats and secondary metabolites; also called phytochemicals are found in smaller range of plants and serve a specific purpose since these have protective properties [19].

Secondary metabolites (e.g. terpenoids) are responsible for the plant's odors while others (e.g. quinones and tannins) for its pigments. Some of these metabolites with bioactive components from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds. Reports indicated that antimicrobial compounds are associated with these phytochemicals. Nutraceuticals are of interest nowadays due to its availability in nature and potentials in promoting health and wellness [20].

Plant metabolites and secondary metabolites of bamboo (kauayan-tinik) leaf were determined and results are shown in Table 4. The ethanolic extract of leaf contains tannins, flavonoids (cyanidin), terpenoids, cardiac glycosides, carbohydrates, and reducing sugar. On the other hand, the leaf does not possess alkaloids, saponins and steroids.

Aakruti et al. pharmacognostical evaluation of various extracts of kawayan tinik suggested that sample leaves yielded alkaloids, carbohydrates, steroids, tannins, glycosides, and flavonoids. The differences of the results with those of similar studies may be due to the methods used in the analysis, place of cultivation, maturity of leaf samples and some other environmental factors (Table 4).

Phytoconstituent	Kawayang tinik leaves		
	Ethanolic extract		
	Trial	Trial	Trial
	1	2	3
Alkaloids:			
a. Mayer's test	-	-	-
b. Dragendorff's test	-	-	-
Saponins (Frothing test)	-	-	-
Tannins ($FeCl_3$ test)	+	+	+
Flavonoids (e.g. Cyanidin) (Shinoda test)	+	+	+
Steroids (Liebermann-Burchard test)	-	-	-
Terpenoids (Salkowski method)	+	+	+
Cardiac Glycosides (Keller-Killiani test)	+	+	+
Carbohydrates (Molisch's test)	+	+	+
Reducing Sugars (Fehling's test)	+	+	+

Table 4: Qualitative metabolites and phytochemical contents of 'kauayan-tinik' leaves ethanolic extracts.

The importance of tannins as a potential antioxidant may be related to its effects in protecting cellular oxidative damage (e.g. lipid peroxidation) [21]. On the other hand, flavonoids are plant metabolites thought to provide health benefits through cell-signaling pathways and antioxidant effects. It was reported that flavonoids may have a part in mechanisms involved in enclosing cell-damaging free radicals and metallic ions. Cardiac glycosides, another phytoconstituent, may increase the output force of the heart and increase its rate of contractions through their action on the cellular sodium-potassium ATPase pump. It may exert a positive inotropic effect on the heart in cardiac failure.

Sensory Evaluation

According to Stone and Sidel, sensory evaluation "evokes, measures, analyzes, and interprets responses to the characteristics of products as perceived by the senses" [22]. It is a means of determining whether product differences can be perceived or whether one product is liked more than another. Sensory evaluation reflects the flavor profile of a product and preferences of the consumers. However, it may be noted that the sensory evaluation results are obtained only through the evaluator's perception. The effect of subjective assumption is sometimes unavoidable, thus measures to prevent or lessen bias should be implemented.

Color is associated with pH, acids and the pigment available in the fruit variety; aroma with volatile compounds such as the alcohol composition, acids, esters and aldehydes produced in the process; flavor with acids, sugars content and organic compounds; astringency with the alcohols produced, texture with the acid concentration and crisp produced by the alcohols. General acceptability is used as the basis for determining the most acceptable treatment for each variety of fruits. The mean scores of the sensory attributes of lipote and bignay wines fortified with 1% and 2% fresh bamboo (‘kawayan-tinik’) leaves were presented in Table 5.

Wine	**Sensory attributes****							
Sample*	**Color**	**Clarity**	**Mouth feel**	**Sweetness**	**Aroma**	**Flavor**	**Off-flavor**	**General Accept-ability**
B1	5.73 ± 0.70a	4.53 ± 0.51a	4.20 ± 0.88a	4.53 ± 0.51a	4.07 ± 0.26a	3.67 ± 0.72b	2.80 ± 0.77a	5.53 ± 0.83a
B2	5.67 ± 0.89a	4.20 ± 0.41b	4.73 ± 0.41a	4.53 ± 0.63a	4.47 ± 0.64a	4.47 ± 0.63a	2.33 ± 0.61a	5.07 ± 0.45a
L1	3.47 ± 0.63b	2.26 ± 0.59c	3.53 ± 0.91b	3.33 ± 0.81b	2.73 ± 1.03b	3.53 ± 0.52b	2.66 ± 0.48a	5.06 ± 0.26a
L2	2.2 ± 0.41c	2.00 ± 0.00c	2.4 ± 0.91c	3.80 ± 1.01b	2.93 ± 0.46b	2.73 ± 0.70c	2.80 ± 0.86a	5.40 ± 0.63a

Table 5: Mean scores and Kruskal-Wallis grouping of the sensory attributes of lipote and bignay wines infused with 1% and 2% fresh bamboo (‘kawayang tinik’) leaves. *B1: Bignay wine with 1% fresh bamboo leaves; B2: Bignay wine with 2% fresh bamboo leaves; L1: Lipote wine with 1% fresh bamboo leaves; L2: Lipote wine with 2% fresh bamboo leaves; **: Average of 15 replications. In each column, means followed by a common letter are not significantly different at 5% level

Range of scores:

Color: 1-Pale Red; 7–Dark red

Clarity: 1–Clear; 7–Cloudy

Mouth feel: 1–Weak; 7-Strong

Sweetness: 1–Dry; 7–Sweet

Aroma: 1–Weak; 7–Strong

Flavor (grassy): 1-Weak; 7-Strong

Off-flavor: 1-Imperceptible; 7-Perceptible

General acceptability: 1–Unacceptable; 7-Acceptable

One of the most important factors shaping consumer perception is the visual appearance of the food products, the primary focus of which is color. Among the wine samples, there is no significant difference between bignay wine variants either infused with 1% (5.73+0.70) or 2% (5.67+0.89) fresh bamboo leaves when it comes to color. However, color change is can be distinguisehd in lipote wine variants upon increase of bamboo leaves from 1% to 2% (Table 5). Generally, the bignay wine variants produced were dark bloody-red, while lipote wine variants were of lighter pinkish color of red hue.

Clarity means clearness or absence of suspending solids in the liquid. In Table 5, the intensity of red color of bignay wines affected the clarity of the samples (B1 4.53+0.51, B2 4.20+0.41) making them darker in hue in comparison with the lipote wine variants which are considerably clearer than bignay wines (L1 2.60+0.59, L2 2.00+0.00). It might be harder to distinguish among samples which one is visually clear if the sample has a very dark hue such as in bignay wine. Accordingly, lipote wine variants are clearer compared to bignay wine variants (Table 5).

Mouthfeel is another important attribute of any alcoholic drinks. The term “mouthfeel” pertains to the sensations that are felt inside the mouth while eating or drinking. Bignay wine variants B1 (4.20+0.88) and B2 (4.73+0.41) had no significant differences when it comes to mouthfeel, while lipote wine variants L1 (3.53+0.91) and L2 (2.4+0.91) were distinguishable from each other. Lipote wine infused with 2% fresh bamboo was found to have the weakest mouthfeel (Table 5).

Sweetness in general, is one of the five basic taste sensations usually pleasing to the taste and typically induced by sugars. Since wine has a sugary constituent both from fruits and the additional sugar input from the processing, sweetness as residual sugar could be the one of the main flavors or taste sensation which could be distinguished in wine beverages. Among the important fruit flavor components in wine our taste buds, aside from sweetness is sourness (acidity) and bitterness as astringency which may come from tannins [23]. Aroma on the other hand, is a strong pleasant smell which usually comes from food or drink. Likewise, aroma of both wines was affected by the level of bamboo leaves. Aroma also can have a sensory effect on the taste of foods wherein fuller aroma may imply more palatable food or beverage items.

In this study, it has been analyzed that bignay variants B1 and B2 which were grouped to be sweetest variants (B1 4.53+0.51, B2 4.53+0.63), also had the stronger aroma than lipote variants L1 and L2. However, it may also be noted that the increased of bamboo from 1% to 2% may seem to have no effect on the sweetness and aroma of both bignay and lipote variants (Table 5).

Flavor is the distinctive taste of something, experienced in the mouth. In this study, it was specified that the flavor of wine be judged as to its grassy flavor since bamboo is a type of grass. Of all the variants, B2 had the strongest grassy flavor B2 (4.47+0.63) white L2 (2.73+0.70) had the least. The increase of bamboo leaves seemed to have an impact to the "grassiness" of wine flavor since variant B2 (4.47+0.63) has been distinguished to B1 (3.67+0.72), similarly L1 (3.53+0.52) to L2 (2.73+0.70). The wine with the strongest grassy flavor was found to be variant B2 (4.47+0.63).

On the other hand, off-flavors which are flaws in food products caused by the presence of undesirable compounds due to chemical changes during food processing, storage, and spoilage by microorganisms. All the wine variants generally had very faint, minor, and not discernible off-flavors ranging from 2.33 to 2.80 out of 7 (Table 5).

General acceptability can be considered for assessing how the product will be received and perceived by the general market through the evaluation of the sample participants [24]. All wine variants are of the same group and were generally acceptable with scores ranging from 5.06 to 5.53 out of 7 (Table 5) [25].

The Kruskal-Wallis Test for the sensory data was provided in Table 6 (Figure 2).

Figure 2: Documentation during sensory evaluation of lipote and bignay wines at DOST-FPRDI, College, Los Banos, Laguna

Sensory attributes	DF	Chi-Square value	Pr > Chi-Square
Color	3	49.99	<.0001**
Clarity	3	50.43	<.0001**
Mouthfeel	3	33.41	<.0001**
Sweetness	3	23.5	<.0001**
Aroma	3	40.42	<.0001**
Falvor	3	28.67	<.0001**
Off-flavor	3	4.54	0.2082 ns
General acceptability	3	5.9	0.1166 ns

Table 6: Kruskal-Wallis test for sensory data**.**

Conclusion

The study aimed to develop the technology of processing carbonated bamboo red wines from lipote and bignay fruits Production of Lipote and Bignay wines adopted the standard method of red wine processing using Saccharomyces ellipsoideus as starter, innovation made was that the process was modified by infusion with fresh bamboo (e.g. kauayan-tinik) leaves during fermentation and carbonation of the finished products.

Fortification with higher level of bamboo leaves exhibited additional increase in both PC and DPPH values; with bignay wines showing higher values than lipote wines for phenolic content. All treatments with bamboo leaves obtained higher inhibition activity compared to the synthetic antioxidant BHT; with lipote wines exhibiting numerically higher values than bignay wines.

The ethanolic extract of bamboo leaf contained tannins, flavonoids (cyanidin), terpenoids, cardiac glycosides, carbohydrates, and reducing sugar. On the other hand, the leaf extract did not have alkaloids, saponins and steroids thus which may indicate that abrupt effects (e.g. hallucinations, pain-relief, poisoning) may be unlikely to happen in the individual's metabolism upon intake of bamboo leaf extracts, although these should be further confirmed in next studies.

Sensory tests for different attributes such as color, clarity, mouth feel (alcohol), sweetness, aroma, flavor, off-flavor and general acceptability of the different treatments using 7-point scale were determined. Results of sensory analysis showed that in comparison to lipote wine variants, bignay wine variants generally had the darker color, lower clarity, stronger mouthfeel. Bignay wine variants were also sweeter and more aromatic than Lipote variants. The addition of bamboo also contributed to the wine's grassy flavor. In terms of general acceptability, all variants were generally acceptable with no significant differences, and only minor or weak off-flavors have been noted.

Recommendations

It is recommended that further study be made on the downstream processing of red wine products such as aging in wine barrels. Shelf life determination, cost analyses and market evaluation may also be studied in the future.

Acknowledgment

The authors wish to acknowledge the food microbiology laboratory, institute of food science and technology, UPLB college of agriculture and food science; DOST-forest products research and development institute; and CS first, agri-industrial, Bayambang, Pangasinan for collaborative work and research funds

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