Physicochemical properties of bignay [*Antidesma bunius* (L.) Spreng.] wine at different stages of processing

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Bignay [*Antidesma bunius* (L.) Spreng.] fruits were used in the preparation of wine. Must and wine samples were obtained at the following stages of processing: must upon dilution with water, after adjustment of sugar content, before addition of wine yeast, before aerobic fermentation, during aerobic fermentation (after 1 and 3 days), and during anaerobic fermentation (end of 1st and 2nd week); and raw wine and aged wine (1st, 2nd, and 6th month). These were analyzed for the following physicochemical properties: pH, total titratable acidity, total soluble solids, total sugars, total reducing sugars, alcohol content and amino-nitrogen. The values obtained ranged from 2.92±0.01 – 3.64±0.03, 0.67±0.03 – 1.10±0.05%, 1.4±0.0 – 21.0 ±0.0 °Brix, 6.89±0.12 – 151.48±0.39 mg glc mL^{-1}, 5.73±0.06 – 96.75±0.82 mg glc mL^{-1}, 0±0 – 12±0% and 12.74±0.00 – 63.70±12.74 mg N L^{-1}, respectively. Results indicated that the changes in the physicochemical properties of the must/wine samples at different stages of bignay wine processing are correlated with each other. A good knowledge of the changes of the physicochemical properties of local wines during processing would contribute to the development of good quality wines, which would be beneficial to local wine manufacturers.

KEY WORDS

bignay, chemistry, food science, must, physicochemical, wine

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INTRODUCTION

In the Philippines, a variety of tropical fruit wines are produced either for home consumption or commercial purposes. However, tropical wines are subjectively perceived to be of inferior quality on the basis of flavor, aroma, bouquet, and color. This may be due to their low sugar content, high acidity, and the presence of microorganisms other than wine yeast. Modifications in the grape wine production, such as pasteurization, amelioration of fruit juices with sugar, and

addition of water, have been applied to tropical fruits to produce acceptable tropical wines.

Bignay [*Antidesma bunius* (L.) Spreng.] is native to the Philippines and often grows in the mountains with a tropical climate. It belongs to the Euphorbiaceae family. It has ovoid-shaped fruits clustered together in a bunch of 30-40 small fruits. It is colored green and turns red to black as it ripens. The fruit has a sour sweet taste when ripe and is commonly used to make jam and wine (Figure 1).

There is a considerable lack of information with regard to the physicochemical properties of these locally manufactured wines. In particular, there has been no report in the literature about the changes in the physicochemical properties at each stage of bignay wine processing. This is the first study that investigates these changes and attempts to explain the biochemical aspects of such changes. Knowing the changes in the physicochemical properties during the manufacture of bignay wine, as well as those of the final product, would allow manufacturers to develop high-quality wines comparable, or even superior, to popular, highly accepted wines.

MATERIALS AND METHODS

Wine processing was conducted at the Food Microbiology Laboratory (FML), Institute of Food Science and Technology (IFST), CA, UPLB, College, Laguna. The analysis of physicochemical properties was done at the Institute of Chemistry, CAS, UPLB, College, Laguna.

Raw materials.

Fully ripe bignay [*Antidesma bunius* (L.) Spreng.] fruits were obtained from the Institute of Plant Breeding, UPLB, College, Laguna. These materials were transported to the FML, IFST, CA, UPLB, College, Laguna. The fruits were then manually sorted to obtain fully ripe, whole fruits devoid of

Figure 1. Bignay fruits clustered together in a bunch of 30-40 small fruits. Each fruit ripens unevenly, so the fruits in a bunch vary in shades of color from greens and yellows to reds and purples.

blemishes. These were then detached from the stem, washed, and drained.

Processing of bignay wine

The bignay wine was prepared using the method of Dizon (2009) as follows (Figure 2).

Must preparation. An amount of 7.5 L of water was added to 2.5 kg of bignay fruits then macerated using a blender (10-speed Osterizer, model 4172-079; Tlalnepantla, Mexico) to produce the must. The sugar content of the must was adjusted from 1.4 to 20 ºBrix by addition of 2.25 kg of refined cane sugar and then mixed thoroughly. About 10% of the must was separated for starter preparation.

Starter preparation. *Saccharomyces bayanus* was obtained from the FML, IFST, CA, UPLB, College, Laguna. The cultures were transferred into potato dextrose agar slants and incubated at 28–30 ºC for 48–36 h. Ten percent of the total volume of the must was placed in an Erlenmeyer flask and plugged with cotton. This was pasteurized in boiling water for 30 min, cooled to 40–45 ºC and inoculated with *S. bayanus*. Fermentation was allowed to take place for 24 h at room temperature. The fermented mixture served as the starter for wine making.

Treatment of the must. Five milliliters of 10% sodium metabisulfite (Univar, AJAX Finechem, Auburn, NSW, Australia) were added per 3.79 L of the prepared must to destroy spoilage microorganisms. The must was distributed into gallon jars. Space was allotted for the addition of starter and the rise of the must during active fermentation. The gallon jars were plugged with clean cotton and allowed to stand for 24 h at room temperature.

Fermentation. The previously prepared starter was added to the treated must. Aerobic fermentation was allowed to take place for 3 d. The cotton plugs were then replaced with fermentation locks and anaerobic fermentation was allowed to

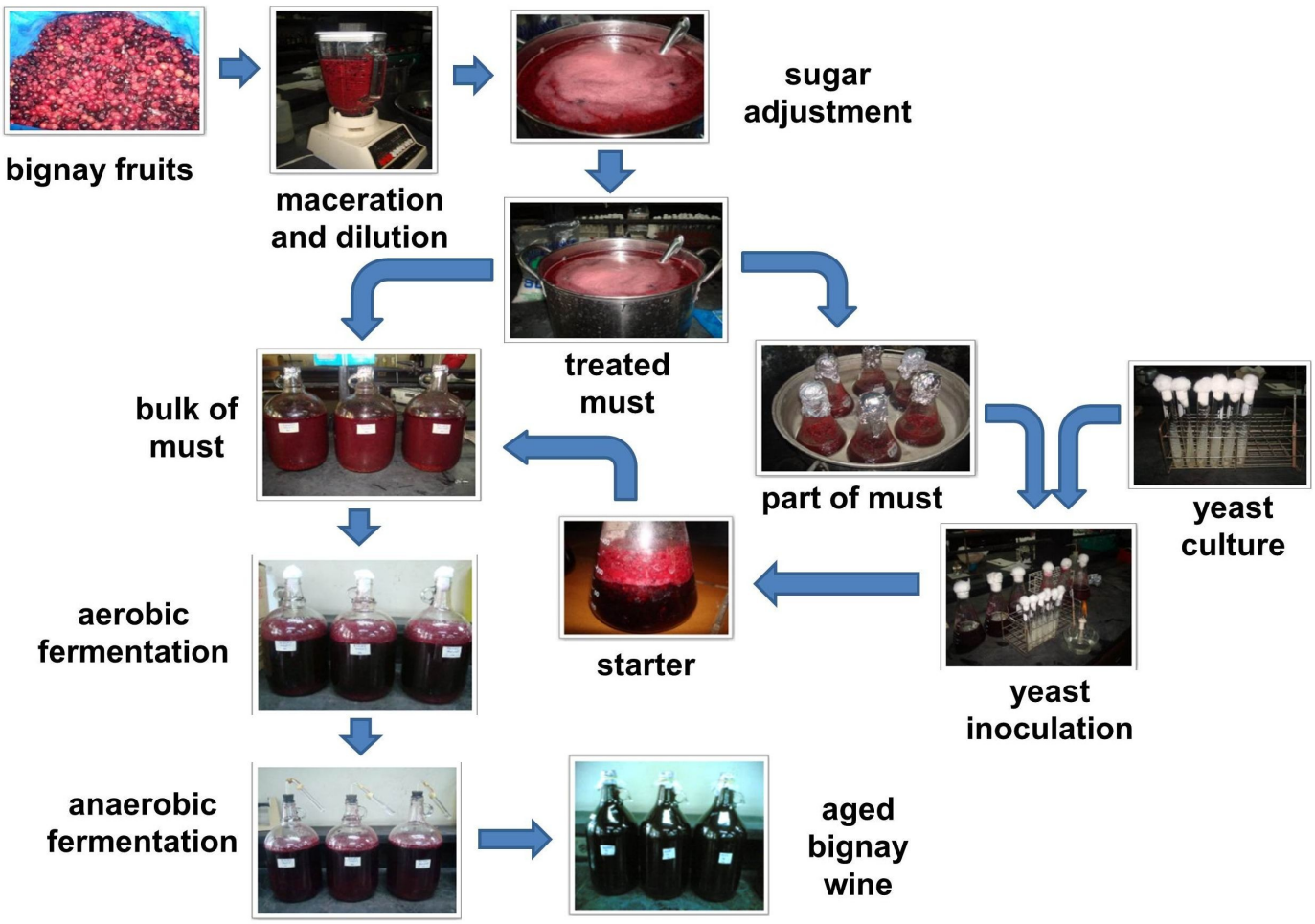

Figure 2. Schematic diagram of the preparation of bignay wine.

proceed for 3 wk.

Harvesting, storing, and aging. The wines were filtered through clean cheesecloth and placed in sterilized gallon jars. Five milliliters of 10% sodium metabisulfite were added per 3.79 L of wine. The gallon jars were covered with polyethylene sheet tied with rubber band and stored at room temperature (20–22 °C) in a dry place for aging.

Analysis of Physicochemical Properties

The analysis of the physicochemical properties was done on the following samples: must upon dilution with water, must after adjustment of sugar content, must before addition of wine yeast, must before aerobic fermentation, must during aerobic fermentation (after 1 and 3 d), must during anaerobic fermentation (end of 1st and 2nd wk), raw wine, and aged wine (1st, 2nd, and 6th mo). All of the assays were performed in three replicates.

pH. The pH of the samples was determined with a CyberScan 1000 pH meter (Eutech Instruments, Singapore) which was calibrated with standard buffer solutions of pH 4.0 and 7.0. An adequate amount of sample was placed in a beaker and its pH was determined at room temperature.

Total Titratable Acidity (TTA). The TTA of the samples was determined using the method of Zoecklein et al. (1999) with some modifications. A CyberScan 1000 pH meter was calibrated with standard buffer solutions of pH 4.0 and 7.0. Twenty milliliters of previously boiled, cooled distilled water (H_2O) were placed in a 100-mL beaker and the pH was adjusted to pH 8.2 using standardized 0.01 N sodium hydroxide (NaOH) (Univar, AJAX Finechem, Auburn, NSW, Australia). One milliliter of wine sample was added into the beaker and the mixture was titrated with standardized 0.01 N NaOH until pH 8.2. The TTA was expressed in terms of tartaric acid (g 100^{-1} mL^{-1}).

Total Soluble Solids (TSS). A hand-held Atago °Brix refractometer was used to determine the TSS of the samples. Two to three drops of sample were placed on the prism of the refractometer then the TSS reading was taken directly and expressed as °Brix.

Total Sugars (TS). The phenol-sulfuric acid method was followed to determine the TS. One milliliter of sample was placed into an acid-washed test tube to which 1 mL of 5% phenol (Merck, Darmstadt, Germany) was added. Five milliliters of concentrated sulfuric acid (H_2SO_4) (JT Baker, Phillipsburg, New Jersey, USA) were added rapidly by direct stream of acid to liquid surface. The mixture was mixed using a vortex mixer and allowed to stand for 10 min. It was placed in a

Table 1. Physicochemical properties of the bignay [*Antidesma bunius* (L.) Spreng.] must/wine samples during processing.

SAMPLE/STAGE	pH	Total Titratable Acidity (% tartaric acid)	Total Soluble Solids (°Brix)	Total Sugars (mg glc mL^{-1})	Total Reducing Sugars (mg glc mL^{-1})	Alcohol Content (%)	Amino-Nitrogen (mg N L^{-1})
Must upon dilution with water	3.64 ± 0.03^{A}	0.76 ± 0.02^{D}	1.4 ± 0.0^{G}	13.51 ± 0.10^{H}	11.34 ± 0.69^{E}	0 ± 0^{H}	36.30 ± 3.93^{C}
Must after adjustment of sugar content	3.50 ± 0.01^{B}	0.67 ± 0.03^{E}	21.0 ± 0.0^{A}	151.48 ± 0.38^{A}	96.75 ± 0.82^{A}	0 ± 0^{H}	63.42 ± 0.00^{A}
Must before addition of wine yeast	3.44 ± 0.01^{C}	0.69 ± 0.01^{E}	20.5 ± 0.0^{AB}	151.14 ± 1.02^{AB}	96.39 ± 4.30^{A}	0 ± 0^{H}	63.42 ± 6.34^{A}
Must before aerobic fermentation	3.41 ± 0.01^{D}	0.72 ± 0.03^{DE}	20.0 ± 0.0^{B}	149.95 ± 0.68^{BC}	95.40 ± 2.02^{A}	0 ± 0^{H}	63.70 ± 12.74^{A}
Must after 1 day of aerobic fermentation	3.36 ± 0.01^{E}	0.76 ± 0.05^{D}	20.0 ± 0.0^{B}	131.49 ± 1.14^{D}	91.84 ± 1.80^{B}	0 ± 0^{H}	61.58 ± 3.68^{A}
Must after 3 days of aerobic fermentation	3.29 ± 0.01^{F}	0.83 ± 0.08^{C}	18.0 ± 0.0^{C}	128.13 ± 0.56^{E}	90.54 ± 1.71^{B}	2 ± 0^{G}	59.45 ± 3.68^{A}
Must after 1 week of anaerobic fermentation	2.96 ± 0.02^{H}	0.94 ± 0.01^{B}	8.5 ± 0.0^{D}	49.15 ± 1.71^{F}	31.99 ± 0.74^{C}	6 ± 0^{F}	57.33 ± 6.37^{AB}
Must after 2 weeks of anaerobic fermentation	2.94 ± 0.01^{H}	0.97 ± 0.00^{B}	7.0 ± 0.0^{E}	28.66 ± 0.48^{G}	16.18 ± 0.58^{D}	8 ± 0^{E}	48.84 ± 3.68^{B}
Raw wine	2.92 ± 0.01^{I}	1.06 ± 0.01^{A}	6.0 ± 0.0^{F}	10.85 ± 0.31^{I}	6.29 ± 0.06^{F}	9 ± 0^{D}	21.23 ± 3.68^{D}
Aged wine (1 month)	2.94 ± 0.02^{HI}	1.09 ± 0.03^{A}	6.0 ± 0.0^{F}	7.70 ± 0.15^{J}	6.36 ± 0.06^{F}	10 ± 0^{C}	14.86 ± 3.68^{D}
Aged wine (2 months)	3.05 ± 0.01^{G}	1.10 ± 0.03^{A}	6.0 ± 0.0^{F}	6.965 ± 0.26^{J}	5.77 ± 0.00^{F}	11 ± 0^{B}	14.86 ± 3.68^{D}
Aged wine (6 months)	3.03 ± 0.01^{G}	1.10 ± 0.05^{A}	6.0 ± 0.0^{F}	6.89 ± 0.12^{J}	5.73 ± 0.06^{F}	12 ± 0^{A}	12.74 ± 0.00^{D}

Values are expressed as the means of 3 replicates. Means in a column followed by the same letter are not significantly different at 5% level of significance using LSD.

water bath at 25- 30 ºC for 10- 20 min. The absorbance was read at 490 nm using a Shimadzu UV-mini 1240 spectrophotometer (Tokyo, Japan). The results were expressed as mg glucose mL^{-1} (fresh weight).

Total Reducing Sugars (TRS). The estimation of TRS was based on the dinitrosalicylic acid (DNS) method of Miller (1959) with some modifications. The DNS reagent was prepared by dissolving 10.6 g of DNS (Ajax Chemicals, NSW, Australia) and 19.5 g of NaOH in 600 mL of distilled water, and gently heated in a water bath at 80 ºC until a clear solution was obtained. Then, 306 g of sodium potassium tartrate tetrahydrate (Kansai rgt corp, Hirikita, Japan), 7.5 mL phenol (Merck, Darmstadt, Germany) (melted at 60 ºC), and 8.3 g of sodium bisulfite (Univar, AJAX Finechem, Auburn, NSW, Australia) were added. The final volume was raised to 1416 mL with H_2O. The solution was filtered and stored at room temperature in a dark bottle.

One milliliter of sample was placed in a test tube and 2.0 mL of DNS reagent were added after which the solution was mixed and covered with a glass marble. The mixture was heated in a boiling water bath for 15 min, cooled to room temperature, and then diluted to 20 mL with distilled H_2O. Absorbance was read at 550 nm using a Shimadzu UV-mini 1240 spectrophotometer (Tokyo, Japan). The results were expressed as mg glucose mL^{-1} (fresh weight).

Alcohol Content (AC). Forty milliliters of sample and 20 mL of distilled water were placed in a round bottom flask. Distillation was done until 30 mL of distillate was collected in a 50-mL graduated cylinder. The distillate was made up to a volume of 40 mL using distilled water. The solution was mixed and cooled to 20 ºC. Percent alcohol was read directly using a hydrometer.

Amino-Nitrogen (AN). The AN content of the samples was determined using the formol titration method of Zoecklein et al. (1999) with some modifications. The sample from TTA analysis (when the solution reached its endpoint) and 1 mL of formalin (Ajax chemicals, NSW, Australia) (previously neutralized with 0.01 N NaOH solution until pH 8.2) were mixed. An unchanged pH after mixing indicated that amino nitrogen was absent. When the pH decreased, titration was continued until pH 8.2 was achieved. The AN was expressed as mg N L^{-1}.

Statistical Analysis

The data for physicochemical properties were reported as the mean ± standard deviation computed from three values. ANOVA using CropStat® statistical analysis software was used to determine the significant differences of each variable at different stages of wine processing. Means separation was done using LSD at 5% level of significance. Pearson product moment correlation analysis (r) using SPSS® version 16 was done to determine strength of the relationships between parameters. Statistical significance was inferred at $P<0.05$ (95%) and $P<0.01$ (99%) level of confidence.

RESULTS AND DISCUSSION

Physicochemical Properties of Bignay Must/Wine Samples During Processing

Bignay fruits were used to produce a red wine. Must and

Table 2. Correlation analysis of the physicochemical properties of bignay [Antidesma bunius (L.) Spreng.] must/wine samples during processing.

VARIABLES	pH	Total Titratable Acidity	Total Soluble Solids	Total Sugars	Total Reducing Sugars	Alcohol Content	Amino-Nitrogen
pH	1	-0.533	0.387	0.617	0.640	-0.852	0.481
	0.000	**	*	**	**	**	**
Total Titratable Acidity		1	0.014	-0.333	-0.346	0.781	-0.129
		0.000	ns	*	*	**	ns
Total Soluble Solids			1	0.921	0.902	-0.425	0.805
			0.000	**	**	**	**
Total Sugars				1	0.995	-0.713	0.843
				0.000	**	**	**
Total Reducing Sugars					1	-0.729	0.834
					0.000	**	**
Alcohol Content						1	-0.608
						0.000	**
Amino-Nitrogen							1
							0.000

ns no significant correlation; * correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed)

wine samples were obtained during processing and analyzed for physicochemical properties. The values are summarized in Table 1.

pH. The pH of the must upon dilution with water was 3.64±0.03 which decreased significantly to 3.44±0.01 before the addition of yeast. This may have been due to the extraction of acidic components from the fruit into the must or to the metabolic activity of organic acids by other microorganisms that were already present in the must. The decrease in pH continued after addition of wine yeast until the first week of anaerobic fermentation with a value of 2.96±0.02, which may be attributed to the production of organic acids from the utilization of sugars by the yeast for its growth. Organic acids such as pyruvic acid are produced during glycolysis, which may be further metabolized into other organic acids such as succinic acid and malic acid in the citric acid cycle, and which are then excreted into the must. The citric acid cycle produces carbon dioxide and water, which leads to the formation of carbonic acid, thus decreasing the pH. There was no significant change in pH from the 1st week to the 2nd week of anaerobic fermentation. Under anaerobic conditions, the pyruvate resulting from glycolysis is decarboxylated to acetaldehyde, which is reduced to ethanol.

The decrease in pH to 2.92±0.01observed during wine harvest may be due to the effect of filtration, which may have led to the extraction of other organic acids from the press residues as reflected by the significant increase in the titratable acidity. No significant change was observed during the 1st month of aging. Perhaps, the production of organic acids had ceased as reflected by the stable TTA values. However, a significant change was observed during the 2nd month, which stabilized until the 6th month with a value of 3.03±0.01. Some components of the wine may have been responsible for its buffering activity.

Total Titratable Acidity. The TTA value of the must upon dilution with water was 0.76±0.02%, which significantly decreased to 0.67±0.03% after adjustment of the sugar content. The decrease in TTA may be due to the masking of the acids by the added sugar. Increasing values until the 1st week of anaerobic fermentation (0.94±0.01%) may be attributed to the uptake of sugars by the yeast, which metabolized the sugars into organic acids (through glycolysis and the citric acid cycle), some of which may have been released into the must. This is reflected by the decrease in the total sugar content of the must.

The organic acids of microbial origin that contribute to the acidity of the must are succinic, keto-acids pyruvic and α-ketoglutaric acid, L(+)-lactic acid, and lesser amounts of other non-volatile acids (Boulton et al. 1996). Keto-acids play a role in the formation of stable wine pigments such as reacting with anthocyanins to form pyranoanthocyanins, which are more stable to oxidative degradation than anthocyanin pigments (Bakker and Timberlake 1997).

No significant change was observed on the 2nd week of anaerobic fermentation. By this time, the yeast had metabolized the sugars into ethanol instead of producing organic acids in the citric acid cycle, thus the change in titratable acidity is very small. Fermentation has little effect on total acidity, but it does increase their chemical diversity. The increased complexity may play a minor role in the development of an aged bouquet (Jackson 2008).

The stable TTA values during aging (1.10±0.05%) may be due to the absence of yeast that had been previously separated prior to aging.

Total Soluble Solids. The soluble solids of musts and sweet wines are composed mostly of sugars. The TSS value is a useful indicator of potential alcohol yield after fermentation and the likelihood of residual sugars remaining (Jackson 2008).

The TSS value of the must after dilution with water was 1.4±0.0 ºBrix, which abruptly increased to 21.0±0.0 ºBrix upon adjustment of sugar content. This was expected since soluble solids of musts are composed mostly of sugars. The decrease in TSS value to 18.0±0.0 ºBrix during aerobic fermentation could be due to the utilization of sugars by yeast to produce organic acids and other metabolites, as well as the production of a little amount of alcohol. *S. bayanus* has the capability to carry out fermentation even in the presence of oxygen, given a high enough concentration of sugar (Serra et al. 2003).

The abrupt decrease in TSS value to 8.5±0.0 ºBrix on the 1st week of anaerobic fermentation, which continuously decreased to 6.0±0.0 ºBrix until the end of anaerobic fermentation, can be attributed to the utilization of the sugars for the production of alcohol. Under anaerobic conditions, the yeast converts the glucose, fructose, and sucrose found in must into ethanol via the process of fermentation. Aging resulted in a stable TSS value of 6.0±0.0 °Brix. By this time, the yeast was already separated from the must, thus, no utilization of sugar occurred as reflected by the insignificant changes in sugar content.

Total Sugars and Total Reducing Sugars. The total sugar and reducing sugar contents of the must upon dilution with water were 13.51±0.10 and 11.34±0.69 mg mL^{-1}, respectively, which abruptly increased to 151.48±0.39 and 96.75±0.82 mg mL^{-1}, respectively, upon sugar adjustment. The increase in both sugar contents was expected, as table sugar was added into the must. There were no significant changes until before aerobic fermentation, which indicated the non-utilization of sugars. There was a slight but significant decrease in both sugar contents after the 1st day of aerobic fermentation, which continued until the 3rd day of aerobic fermentation. The sugars may have been metabolized in glycolysis and the citric acid cycle for the production of metabolites required for growth and reproduction of the yeast.

An abrupt decrease to 49.15±1.7 and 31.99±0.74 mg mL^{-1} in total and reducing sugars, respectively, was observed during the 1st week of anaerobic fermentation, which continually decreased until the end of anaerobic fermentation with values of 10.85±0.31 and 6.29±0.06 mg mL^{-1}, respectively. The yeast may have degraded the simple sugars into ethanol and carbon dioxide, glycerol, aldehydes, lactic acid, and succinic acid. Aging resulted in stable total and reducing sugar contents with final values of 6.89±0.12 and 5.73±0.06 mg mL^{-1}, which indicated that the sugars were no longer used for metabolic purposes as the yeasts were already separated from the wine.

Alcohol Content. The alcohol content of 0±0% of the must upon dilution of water until the 1st day of aerobic fermentation reflects the absence of alcohol. The slight increase in alcohol content to 2±0% on the 3rd day of aerobic fermentation can be attributed to the ability of *S. bayanus* to ferment sugar to alcohol even in the presence of oxygen as mentioned earlier.

There was a further increase in alcohol content to 9±0% until the end of anaerobic fermentation, which can be attributed to the utilization of sugars to produce alcohol. Under winemaking conditions, ethanol, and CO_2 are the major products of alcoholic fermentation of sugars by *Saccharomyces* species. Ethanol, methanol, and polyalcohols are formed in the wine during fermentation. Ethanol is crucial to the stability, aging, and sensory properties of wine (Jackson 2008). The reduction of pyruvate to ethanol sustains the continued operation of glycolysis under anaerobic conditions. The increasing alcohol content progressively limits the growth of microorganisms.

It will be noted that the alcohol content of the wines continued to increase to 12±0% until the 6th month of aging. This was not expected, as the yeasts responsible for alcohol production were supposed to have been completely separated from the wine. It is possible that some microorganisms may have been able to pass through the cheese cloth during filtration and have survived even in the presence of the antimicrobial agent, sodium metabisulfite. The continued increase in alcohol content may be due to the further reduction of acetaldehyde into ethanol, as there were no significant changes in the sugar and amino-nitrogen content in the aged wines.

Amino-nitrogen. The amino-N content of the must after dilution with water was 36.30±3.93 mg N L^{-1}, which increased to 63.42±0.00 mg N L^{-1} after adjustment of the sugar content. This may be due to the release of nitrogenous compounds upon stirring and was facilitated by diffusion. Because of the added sugar, water along with other cell materials are drawn out into the bulk of the mixture (must). There were no significant changes until the 2nd week of anaerobic fermentation. This may suggest that the yeast preferred the intake of carbohydrates over the amino acids for its growth, as reflected by the decreasing amounts of sugar during these stages, or maybe because amino acids can be converted to related amino acids, such as the interconversion between cysteine and methionine (Bisson 1991). However, a significant decrease in amino-N content was observed from the aerobic (57.33±6.37 mg N L^{-1}) to anaerobic (48.84±3.68 mg N L^{-1}) fermentation, which may indicate that by this time the yeast needed the amino acids for the sustenance of its existence because the levels of sugar had largely decreased. A smaller subset of amino acids can be completely degraded releasing ammonia, which can then be used in protein biosynthesis (Perez-Zuñiga et al. 1997). Amino acids utilized as nitrogen sources yield organic acids of the citric acid cycle (Boulton et al. 1996). There was no significant change in amino-N content during aging with a final value of 12.74±0.00 mg N L^{-1}, which may be attributed to the absence of yeast in the wines.

Correlation of Physicochemical Properties of Must/Wine Samples

Correlation analysis of the samples (Table 2) revealed that the pH of samples had a strongly positive correlation with TSS ($r=0.387$, $P<0.05$) and a very strongly positive correlation ($P<0.01$) with AN ($r=0.481$), TS ($r=0.617$) and TRS ($r=0.640$), but had a highly negative correlation ($P<0.01$) with TTA ($r=-0.533$) and AC ($r=-0.852$). These signify that the pH of the samples was very much influenced by the levels of soluble solids such as sugars, as well as the available amino acids. Sugars and amino acids are metabolized by yeast for glycolysis and the citric acid cycle, producing organic acids such as pyruvic, succinic, and α-ketoglutaric acids (Boulton et al. 1996). In turn, alcohols, predominantly ethanol, are produced through utilization of sugars by the yeast during anaerobic fermentation and in small amounts during aerobic fermentation (Jackson 2008).

TTA of the samples had a highly significant ($P<0.01$) and positive correlation with AC ($r=0.781$), a significant and negative correlation with TS ($r=-0.333$) and TRS ($r=-0.346$), but was not significantly correlated with TSS and AN. These signify that high levels of organic acids resulted from the utilization of sugars. The level of pyruvic acid produced during aerobic fermentation may have affected the level of ethanol produced during anaerobic fermentation. The reduction of pyruvate to ethanol may have sustained the continued operation of glycolysis under anaerobic conditions.

The TSS of the samples was highly significant ($P<0.01$) and positively correlated with TS ($r=0.921$), TRS ($r=0.902$) and AN ($r=0.805$), but was negatively correlated with AC ($r=-0.425$). This may signify that the soluble solids were primarily composed of sugars. The fermentable sugar content of must accounts for 90 to 95% of the total soluble solids (Zoecklein et al. 1999). Increase in alcohol content during anaerobic fermentation, which may be attributed to the utilization of sugars, would result in a decrease in TSS.

The TS of the samples had strongly significant ($P<0.01$)

and positive correlations with TRS (r=0.995) and AN (r=0.843) but was negatively correlated with AC (r=-0.713). TRS had a highly significant (P<0.01) and positive correlation with AN (r=0.834), but was negatively correlated with AC (r=-0.729). AC had a highly significant (P<0.01) and negative correlation with AN (r=-0.608). These signify that the level of alcohol increased as the sugars and amino acids were metabolized by the yeast to form pyruvate during glycolysis, which was then converted to alcohol during fermentation. These signify that the sugar content of the must was composed mainly of reducing sugars like glucose and fructose, which were utilized by the yeast during fermentation. Yeast growth continues until assimilable nitrogen (e.g. amino acids) is depleted and, thus, affects the rate and progress of fermentation (Geoffrey et al. 2003).

CONCLUSION

The results of the study showed that the changes in the physicochemical properties of the must/wine samples at different stages of bignay wine processing are correlated to each other. It may suggest that the quality of wine depends on the initial chemical composition of the must. However, the identity and structure of the chemicals present in the must and wine samples should be further studied, in order to gain a better understanding of the development of a high-quality local wine.

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CONFLICTS OF INTEREST

None

CONTRIBUTION OF INDIVIDUAL AUTHORS

Ma. Desiree Belina-Aldemita, as lead author, prepared the draft and finalized the writing of this article for publication. This paper is part of her MS thesis. She was involved in the preparation of the bignay wine, sampling, physicochemical analyses, and interpretation of the results.

Dr. Veronica C. Sabularse is the thesis adviser of the lead author. She was also involved in the interpretation of the results, preparation of the draft, and finalization of the manuscript.

Dr. Erlinda I. Dizon is a panel member of the advisory committee of the lead author. The lead author worked in her laboratory during the preparation of the bignay wine and used her method of wine preparation.

Dr. Wilma A. Hurtada and Dr. Mary Ann O. Torio were panel members of the advisory committee of the lead author.

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