STUDIES ON THE TRADITIONAL SUGARCANE WINE (BASI) PRODUCTION IN THE PHILIPPINES

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Studies were made to document the traditional process of preparation of sugarcane wine ("basi") from the different areas and evaluate chemically the products produced by the different methods.

There were three general methods of preparing "basi", namely: the La Union, Ilocos and Pangasinan. Two types of "basi" were made: the sweet or "basi babae" (29 to 33° Brix) and the drier and bitter type or "basi lalake" (27 to 28° Brix).

Variations among the methods were observed in the preparation, additives used and chemical composition of the resulting wines. Based on the studies, La Union "basi" was the best in chemical analysis. The wines produced have the highest alcohol (14.82%), lowest reducing sugar (0.25%), acidity (5.36 ml of 0.1N NaOH/10 ml) and polyphenol content (182 mg/100 ml) as compared with those produced in the Ilocos Region and Pangasinan. Similarly, great variations in reducing sugar, acidity, alcohol and polyphenol contents between the "basi lalake" and "basi babae" were obtained.

INTRODUCTION

Traditional fermented foods are some of the oldest sources of survival by mankind. Fermentation as a means of food preservation, however, is still the least understood (Pederson, 1971). Knowledge on the role of microorganisms and the physical and chemical changes that occur in fermentation are very necessary for the improvement of the process developed in the homes. The methods based on the careful observation of certain individuals gradually improved. But because the experiment done are mostly on a trial and error ones, the process is not yet standardized.

In the Philippines, there are several traditional fermented foods such as fermented fish sauces ("bagoong" and "patis"), rice wine ("tapuy"), "nata" a product of the growth of Acetobacter;

acetii subsp. xylinum on sugary medium, fermented shrimp ("alamang"), fermented shrimp plus steamed rice ("balao-balo"), vinegar ("suka"), coconut and other palm wine ("tuba"), distilled coconut wine ("lambanog"), fermented rice cake ("puto"), fermented mustard leaves ("bungong mustasa") fermented fish ("bungong isda") and sugarcane wine ("basi").

The quality of "basi" produced in the different "basi" producing areas cannot be ascertained due to the following factors: (1) non-standardized process of preparation (the methods used vary from one maker to another), and (2) unknown microorganisms involved in the fermentation process.

No extensive studies have been done on the following aspects of "basi" making: methods of preparation, microorganisms of the different starters, and the changes that occur during fermentation. This study, therefore, was conducted to accomplish the following general objectives:

1. To survey different "basi" producing areas and observe the traditional methods of preparation.
2. To collect and analyze chemically samples of the different starters used in the preparation and the products derived from the different processes.

STUDY I

Evaluation of the Traditional Sugarcane Wine Production

"Basi" is one of the oldest traditional alcoholic beverages in the Philippines. "Basi" making is a promising industry in the Philippines. This stems from the fact that sugarcane is widely cultivated on a larger scale. For the crop year 1979 to 1980 alone, the land devoted to sugarcane production was 442,201.84 ha yielding a total production of 22,489,847.328 metric tons (Philsocon, 1980). This solid production base should encourage enterprising people to engage in "basi" making.

Sugarcane is a good base for any fermentation product due to its chemical properties. The cane analysis showed that it contains sucrose ranging from 9.6% to 14.90 percent; and fiber, from 10.40 to 14.81 percent (Ocampo and Fandialan, 1975). The crushed juice contains soluble solids ranging from 16.63 to 22.64° Brix.

Preparation of sugarcane wine ("basi").

There are three general methods of preparing "basi", namely: the La Union, Ilocos Region and Pangasinan methods. All these methods utilize sugarcane juice obtained by crushing one year old sugarcane stalks between the wooden or iron rollers provided with a long pole tied to a carabao.

Two types of "basi" are produced: the sweet or "babae" (for women) and the dry and bitter or "lalake" (for men). Their difference lies in the concentration of sugarcane juice ("babae", 29 to 33° Brix and "lalake", 27 to 28° Brix) and the additives added such as "tañgal" (Cerioips tagal (Perr. C.B. Rob.) bark, green guava (Psidium guajava Linn.) leaves, duhat (Syzgium cummuni Linn.) bark, and fruits, bark and leaves of "samac" (Macharanga tanarius Linn. or M. grandifolia Linn.).

A. La Union Method.

The La Union method is used specifically in the town of Naguillan. It consists of the preparation of "bubod" or starter, and the 24-hour "binubudan" (steamed rice plus starter), boiling sugarcane juice, and the additives such as the one year old duhat bark, "tañgal" bark and green guava leaves.

The flow diagram for the preparation of "basi" using the La Union method is shown in Figure 1.

B. Ilocos Region Method.

Sugarcane wine is prepared in Ilocos Norte, specifically in Laoac City, and in the towns of Vintar, Sarret, Piddig and Batac, and in Ilocos Sur, in the towns of Bantay and Vigan.

The Ilocos method differs from the La Union method on the starter or inoculum and the additives used.
The flow diagram of the Ilocos Region method of preparing sugarcane wine is shown in Figure 2.

C. Pangasinan Method.

The “basi” industry in Pangasinan is not as popular as those in La Union and Ilocos Region. Most of the people preparing it reside in Binalonan, Pangasinan. The method used is similar to that of the Ilocos region.

The flow diagram of the Pangasinan method of preparing sugarcane wine is shown in Figure 3.

D. Discussions

Based on the studies made on the three general methods of preparing “basi”, the main difference is the source of microorganisms used in the fermentation of sugarcane juice (Table 1). In the La Union method, the “bubod” serves as the inoculum which is normally activated for 24 hours. The Ilocos Region and Pangasinan methods utilize “samac” as source of microorganisms. The former employ “samac” leaves and fruits plus cardis seeds and rice grains, while the latter uses only “samac” fruits. The microorganisms present in the inocula used in Ilocos Region and Pangasinan vary, depending on the environmental conditions and the microflora of the environment where the inocula was collected. On the other hand, the La Union method has approximately predetermined type of microorganisms although the presence of undesirable microorganisms depend on the sanitary conditions during the “bubod” preparation. This explains why the quality of “basi” cannot be ascertained.

Additives, which serve as flavor-enhancing and bacteriocidal, also differ among the methods. In La Union, green guava leaves, duhat and “tañgal” bark are added. In Pangasinan “samac” bark and fruits were used. The former was added during the concentration stage of the sugarcane juice, while the latter after cooling the boiled sugarcane juice. The “samac” bark and leaves used in Ilocos Region are added after the boiled sugarcane juice cooled.

Slight differences were obtained in the sugar concentration, fermentation period,
percent alcohol, residual sugar (°Brix) and yield (%) among the three methods of preparing sugarcane wine.

STUDY II.

Chemical Composition of Sugarcane Wine and Additives

MATERIALS and METHODS

A. Collection

Six places were selected as sampling areas for the chemical analysis of “basi” based on differences on their preparation, inocula used, fermentation and aging, environmental conditions and the popularity of the industry in the particular place. These places were Barangay Lioac, Naguilian, La Union; San Mateo, Ilocos Norte; Piddig, Ilocos Norte; Barangay 4, Sarrat, Ilocos Norte; Bantay, Ilocos Sur; and Barangay Clil, Binalonan, Pangasinan. One year old “basi” were collected for two consecutive production seasons to be able to compare the quality of the products from these different “basi” producing areas.

B. Analytical Methods

Chemical analyses of the samples were done based on the standard methods of analysis of the Association of Official Analytical Chemists (1970).

1. Determination of pH. The pH of the finished wines were measured using the Beckman Zeromatic II pH meter.

2. Determination of Total Titratable Acidity. A five millimeter sample was diluted with distilled water to 150 ml and degassed. Carbon dioxide was removed by heating the diluted sample to incipient boiling for 30 minutes. The flask was swirled and allowed to cool. The diluted sample was titrated with a standard 0.1N NaOH up to pH 8.5 using a pH meter to record the pH. The total titratable acidity is expressed as milli-equivalent acid using the formula:

\[ \text{milliequivalent acid} = \frac{N_b \times V_b}{W} \]

where: 
- \( N_b \) = normality of base 
- \( V_b \) = volume of NaOH used

Figure 2. Ilocos method of preparing “basi.”
3. **Determination of Total Soluble Solids.** This was determined using a hand refractometer.

4. **Determination of Amino Nitrogen.**
   The sample was titrated with 0.1N NaOH up to pH 8.5 as in the determination of total titratable acidity. Five ml of formalin (pH 4.5) was added and the solution stirred. With constant stirring, the solution was back titrated with 0.1N NaOH to bring the pH back to 8.5. Computation of amino nitrogen in mg% was made using the formula:

\[
\text{Amino Nitrogen (mg \%) } = \frac{N \times V \times 14,007}{100 \times \text{Volume of sample}}
\]

where:

\[N = \text{normality}\]
\[V = \text{volume of NaOH used}\]

5. **Determination of Alcohol Content.**
   The percent alcohol was determined using an ebulliometer. The boiling point of water was first determined by pouring 50 ml of distilled water into the boiler while the condenser jacket was filled with distilled water. The point at which the temperature becomes stationary is the boiling point of water. The boiler was emptied of water and rinsed using the sample. Fifty ml of the wine sample was poured into the boiler. The procedure then is the same as in finding the boiling point of water. The corrected boiling point of water was subtracted from the observed boiling point of the sample to get the corrected boiling point of the latter. Using the Lefco Ebulliometer Table, the corrected boiling point of the sample was located on the left hand column and the alcohol percentages by volume and by weight were found.

6. **Determination of Reducing Sugar.**
   The Somogyi Method was used in these determinations. The following reagents were prepared: Reagents A, B, C, D, and E.

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**Table 1. Differences in the methods of “basi” preparations in La Union, Ilocos Region and Pangasinan.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>La Union</th>
<th>Ilocos Region</th>
<th>Pangasinan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial sugar</td>
<td>28 to 33</td>
<td>27 to 30</td>
<td>27 to 29</td>
</tr>
<tr>
<td>concentration (°Brix)</td>
<td>“bubod”</td>
<td>“samac” fruits</td>
<td>“samac” fruits</td>
</tr>
<tr>
<td></td>
<td>“binubudan”</td>
<td>and leaves, cardis seeds</td>
<td>and rice</td>
</tr>
<tr>
<td>Fermentation period</td>
<td>2 months</td>
<td>1 ½ months</td>
<td>1 ½ months</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>12-15</td>
<td>10-13</td>
<td>12-13</td>
</tr>
<tr>
<td>Residual sugar (°Brix)</td>
<td>8-13</td>
<td>10-13</td>
<td>10-13</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>70</td>
<td>60</td>
<td>65</td>
</tr>
</tbody>
</table>
Reagent A:
Sodium potassium tartrate 90 g
Na₂₃PO₄·12H₂O 225 g
CuSO₄·5H₂O 30 g
KIO₃ 3.5 g

These chemicals were heated with distilled water to dissolve and then water was added to make one liter.

Reagent B:
K₂Cr₂O₇·H₂O 90 g
KI 40 g
Distilled water 1 L

Reagent C:
2N Sulfuric acid

Reagent D:
Na₂S₂O₃·7H₂O 24.9 g
Na₂CO₃ 0.2 g
Distilled water 1 L

Reagent E:
1% Starch solution

Pipette 10 milliliter of the sample into a 125 milliliter Erlenmeyer flask. Add ten milliliter of Reagent A and boil for exactly three minutes in the water bath. If after boiling with Reagent A the solution turns very red, dilute the sample further with distilled water until the solution no longer turn red upon boiling. Allow the solution to cool and add ten milliliter each of Reagents B and C. Mix and titrate with Reagent D to a light blue endpoint using Reagent E as the indicator.

The percent reducing sugar is calculated using the following formula:

\[
\% \text{ Reducing Sugar} = \frac{(V_{\text{blank}} - V_{\text{sample}}) \times 2.898 \times F}{\text{DF}} \times \frac{1}{1000}
\]

where:

F = factor for Na₂S₂O₃
DF = dilution factor

To get the factor of Na₂S₂O₃·5H₂O solution, place ten milliliter of 0.1N oxalic acid in 125 milliliter Erlenmeyer flask and titrate with 0.1N KMnO₄. Then, pipette 20 milliliter of 0.1N KMnO₄ and titrate with Na₂S₂O₃·5H₂O

Calculate \( F_{\text{Na₂S₂O₃·5H₂O}} \) as follows:

\[
F_{\text{Na₂S₂O₃·5H₂O}} = \frac{0.1N \text{ oxalic acid}}{(10 \text{ ml})(1.0033)}
\]

7. Determination of Crude Protein.

The protein content of the sample was determined using the Modified Micro Kjeldahl method. Place weighed sample (0.1 g) wrapped in a piece of filter paper in 100 ml Kjeldahl flask. Add approximately 0.5 g selenium mixture and 4 ml concentrated sulfuric acid. Place flask in an inclined position in a fume hood and heat gently, rotating the flask until the solids are dissolved. Occasionally shake to prevent over-heating and drying up of sample. The flame should be adjusted so that it will strike only the bottom of the flask. Too high a flame will cause decomposition and losses of nitrogen. Continue heating until the solution becomes colorless. Heat for another 15 minutes.

Cool, transfer contents of the flask to sample flask of distillation set-up. Wash neck and sides of flasks three times with small amounts of distilled water and add the washings to sample in distillation set-up. Make sure that all connections are tight and the tip of the condenser immersed in a receiver (125 ml Erlenmeyer flask) containing 10 ml of acid depending on the nitrogen content of the sample.

Introduce through a funnel about 15 ml of NaOH-Na₂S₂O₃ solution to the sample and steam distill (Note: Selenium mixture used as catalyst contains Hg which forms a complex with NH₃. This complex is not readily decomposed by alkali and as a result complete distillation of NH₃ is not possible. Hg is therefore precipitated as HgS with thiosulfate before distillation). When enough distillate had been collected (about 75 ml), lower the receiving flask, wash tip of condenser with distilled water from washing bottle and continue distilling for one minute more. Rinse inside of condenser before stopping.

Titrate the contents of the receiver with standard 0.1N HCl to the first appearance of violet color.

Run a blank using all the same amount of reagents and the size of filter paper used for all the sample to correct for any nitrogen present. The crude protein is calculated as follows:

\[
\% \text{Crude Protein} = \frac{(V_{\text{sample}} - V_{\text{blank}}) \times N_{\text{HCl}} \times \frac{14}{1000} \times 6.25 \times \frac{1000}{\text{volume of sample}}}{\text{weight of sample}}
\]

8. Determination of Ash. Place the crucible with the sample from the moisture determination in a muffle furnace. Ignite to 650°C. Cool to about 50°C before placing in a desiccator. Cool to room temperature and weigh (Note: temperature at 650°C should be maintained for two hours). Reignite for another 30 minutes and reweigh. Repeat ignition until there is no more loss in weight. Calculate percent ash as follows:

\[
\% \text{Ash} = \frac{(\text{wt. of crucible + ash}) - (\text{wt. of tared crucible}) \times 100}{\text{Weight of sample taken}}
\]

9. Determination of Polyphenols. The method of Folin-Dennis was followed. Ground samples were extracted by adding water and boiled in water bath for 30 minutes. After boiling, the extract was filtered with a vacuum filter. The volume of the filtrate was concentrated using a vacuum evaporator at 55°C. The concentrated samples were spotted to TLC plastic cellulose plate (without fluorescent indicator) and with
0.1 mm layer thickness then dry. The TLC plates were placed in the developing tank, first solvent is 2% acetic acid solution and the second solvent is butanol: acetic acid: water (4:1:2:2). The chromatogram was developed using K$_3$Fe (CN)$_6$–FeCl$_3$ solution (1:1).

The quantitative analysis of the polyphenols was done by diluting the samples to 1/1, 1/10, 1/50, 1/100, and 1/200. Five ml of the diluted samples were placed in volumetric flasks and then 1 ml of Folin reagent was added. Mix and then leave the mixture for one hour. After one hour read in Spectrophotometer using 700 or 760 nm. The standard used was D-catechin.

The determination of the organic acid was done using Carboxylic Acid Analyzer S-30. The samples were deproteinized using 10% perchloric acid for one hour and then centrifuged at 3,000 rpm for 30 minutes. The supernatant was injected to Carboxylic Acid Analyzer S–30 with the following conditions: Column 30 x 1000 mm, Packing, Anion-exchange resin (8%) 11-14 um; Column temperature: 50°C; Eluent: 0.2N HCL, Flow rate: 0.13 ml/min. Pump 1 (30-36 lbs/sq. in); Pump 2 (11-23 lbs/sq. in); Pump 3 (13-22 lbs/sq. in) and Pump 4 (11-20 lbs/sq. in). The amount of organic acids present in the samples were calculated based on the peaks formed. The standard organic acids used were glutamic, lactic, acetic, pyruvic, malic, proplionic, citric, succinic and alpha-ketoglutaric.

The different samples of "basi" were prepared and then free amino acids were analyzed using the Amino Analyzer JEOL-GAH using lithium citrate solution at pH 2.2 as buffer. Standard amino acids were used in calculating the concentration of free amino acids in the samples.

RESULTS AND DISCUSSION

A. Chemical composition of sugarcane wine.

Table 2A and 2B showed the chemical composition of the two types of "basi." There were great variations on reducing sugar, alcohol and polyphenol contents between the "basi babae" (for women) and "basi lalake" (for men) sampled. The "basi babae" has higher reducing sugar than "basi lalake" on all samples analyzed. This is due to the higher amount of sugar in the initial mixture which the yeast could no longer convert to alcohol.

Table 2A. Chemical composition of "basi babae" samples in Naguillian (La Union), San Mateo (Ilocos Norte), Piddig (Ilocos Norte), Sarrat (Ilocos Norte), Bantay (Ilocos Sur) and Binalonan (Pangasinan).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Naguillian (La Union)</th>
<th>San Mateo (Ilocos Norte)</th>
<th>Piddig (Ilocos Norte)</th>
<th>Sarrat (Ilocos Norte)</th>
<th>Bantay (Ilocos Sur)</th>
<th>Binalonan (Pangasinan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar (%)</td>
<td>13.25</td>
<td>13.00</td>
<td>13.50</td>
<td>13.40</td>
<td>13.62</td>
<td>13.00</td>
</tr>
<tr>
<td>pH</td>
<td>3.45</td>
<td>3.33</td>
<td>3.45</td>
<td>3.50</td>
<td>3.40</td>
<td>3.60</td>
</tr>
<tr>
<td>Acidity</td>
<td>(0.1N NaOH/10 ml)</td>
<td>5.54</td>
<td>4.80</td>
<td>7.78</td>
<td>6.00</td>
<td>5.90</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>12.85</td>
<td>12.00</td>
<td>10.85</td>
<td>11.92</td>
<td>10.95</td>
<td>12.96</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.33</td>
<td>0.37</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Polyphenol (mg/100 ml)</td>
<td>148</td>
<td>252</td>
<td>224</td>
<td>256</td>
<td>238</td>
<td>197</td>
</tr>
</tbody>
</table>

1Average of 3 samples per basi sampling.

Table 2B. Chemical composition of "basi lalake" samples in Naguillian (La Union), San Mateo (Ilocos Norte), Piddig (Ilocos Norte), Sarrat (Ilocos Norte), Bantay (Ilocos Sur) and Binalonan (Pangasinan).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Naguillian (La Union)</th>
<th>San Mateo (Ilocos Norte)</th>
<th>Piddig (Ilocos Norte)</th>
<th>Sarrat (Ilocos Norte)</th>
<th>Bantay (Ilocos Sur)</th>
<th>Binalonan (Pangasinan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar (%)</td>
<td>8.25</td>
<td>10.50</td>
<td>9.75</td>
<td>10.25</td>
<td>10.25</td>
<td>10.50</td>
</tr>
<tr>
<td>pH</td>
<td>3.25</td>
<td>3.20</td>
<td>3.26</td>
<td>3.21</td>
<td>3.18</td>
<td>3.40</td>
</tr>
<tr>
<td>Acidity</td>
<td>(0.1N NaOH/10 ml)</td>
<td>5.36</td>
<td>5.50</td>
<td>7.36</td>
<td>5.64</td>
<td>5.56</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>14.82</td>
<td>13.25</td>
<td>12.92</td>
<td>13.02</td>
<td>12.75</td>
<td>13.60</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.33</td>
<td>0.32</td>
<td>0.32</td>
<td>0.35</td>
<td>0.34</td>
<td>0.37</td>
</tr>
<tr>
<td>Polyphenol (mg/100 ml)</td>
<td>182</td>
<td>291</td>
<td>278</td>
<td>298</td>
<td>282</td>
<td>221</td>
</tr>
</tbody>
</table>

Average of 3 samples per basi season planting.
The Piddig samples were the most acidic followed by the samples from Sarrat, Bantay and San Mateo. There was a slight difference between the Pangasinan and Naguilian samples although the former had lower acidity. These differences in the acid content can be attributed to the presence of acid-forming microorganisms. Adaptive acidity of wines are almost about 0.1-0.2 (for lactic acid) but Pangasinan samples had a range of 2.5-2.62 total acid.

Slight differences on ash and pH were observed among the samples analyzed from different localities.

### B. Polyphenol composition of sugar-cane wine.

Polyphenol concentrations obtained in the samples showed that "basi" prepared using "samac" as additives were higher in Ilocos (from 224 to 298 mg/100 ml) as compared with the lower values in Pangasinan samples (197 to 221 mg/100 ml) so that the wine is less astringent than the other samples. These variations were due to the different amount and kinds of additives containing polyphenols which were added to the fermenting mixture. In the Pangasinan method of preparing "basi" the "samac" leaves were not used.

### C. Organic acid composition of sugar-cane wine.

Analysis of the organic acid content of the samples, using Carboxylic Acid Analyzer is shown in Table 3. The samples analyzed were taken from Barangay Lioac, Naguilian, La Union (A), Barangay San Mateo, Laoag City (B), Barangay Cili, Binalonan, Pangasinan (C) and Piddig, Ilocos Norte (D).

The organic acids found present at varying concentrations were lactate, acetate, pyruvate, malate, propionate, citrate and succinate. Of the different acids, pyruvate recorded the highest values (103.1 to 175 mg/100 ml) on all the samples except for the Piddig sample (54 mg/100 ml). On the other hand, the Piddig sample contained the highest amount of lactate (124.8 mg/100 ml), acetate (147 mg/100 ml) and succinate (104 mg/100 ml) acids. Trace amounts of alpha-ketoglutarate was obtained from all the samples.

### D. Amino acid composition of sugar-cane wine.

Free amino acid analysis of "basi" samples using the Amino Acid Analyzer J-EOL-GAD is shown in Table 4. The "basi" samples collected from the different localities likewise showed variations. The samples consisted of the following: alanine (trace to 0.05 mg/100 ml), arginine (trace to 1.98), aspartic acid (0.02 to 1.70), glutamic acid (0.28 to 1.82), glycine (0.06 to 0.86), histidine (trace to 0.35), isoleucine (0.12 to 0.75),

### Table 3. Organic acids composition of the "basi" samples (mg/100 ml).

<table>
<thead>
<tr>
<th>Organic Acid</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>75.0</td>
<td>88.8</td>
<td>63.2</td>
<td>124.8</td>
</tr>
<tr>
<td>Acetate</td>
<td>53.9</td>
<td>79.9</td>
<td>48.3</td>
<td>147.0</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>119.6</td>
<td>103.1</td>
<td>175.5</td>
<td>54.0</td>
</tr>
<tr>
<td>Malate</td>
<td>14.6</td>
<td>15.4</td>
<td>18.8</td>
<td>43.0</td>
</tr>
<tr>
<td>Propionate</td>
<td>Trace</td>
<td>19.5</td>
<td>40.4</td>
<td>Trace</td>
</tr>
<tr>
<td>Citrate</td>
<td>2.9</td>
<td>Trace</td>
<td>89.9</td>
<td>Trace</td>
</tr>
<tr>
<td>Succinate</td>
<td>63.3</td>
<td>61.5</td>
<td>69.6</td>
<td>104.0</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
</tbody>
</table>

### Table 4. Concentration of free amino (mg/100 ml) in "basi".

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>2.05</td>
<td>1.26</td>
<td>1.93</td>
<td>Trace</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.98</td>
<td>0.13</td>
<td>0.41</td>
<td>Trace</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1.70</td>
<td>0.87</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Cystine</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.60</td>
<td>1.82</td>
<td>2.63</td>
<td>0.28</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.86</td>
<td>0.31</td>
<td>0.57</td>
<td>0.06</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.35</td>
<td>Trace</td>
<td>0.12</td>
<td>Trace</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.75</td>
<td>0.30</td>
<td>0.35</td>
<td>0.12</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.39</td>
<td>0.58</td>
<td>0.60</td>
<td>0.06</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.36</td>
<td>0.41</td>
<td>0.61</td>
<td>0.09</td>
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<tr>
<td>Methionine</td>
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<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.50</td>
<td>0.34</td>
<td>0.31</td>
<td>Trace</td>
</tr>
<tr>
<td>Proline</td>
<td>1.66</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Serine</td>
<td>0.99</td>
<td>0.50</td>
<td>0.48</td>
<td>0.05</td>
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<tr>
<td>Threonine</td>
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<td>0.46</td>
<td>0.27</td>
<td>0.06</td>
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<tr>
<td>Tyrosine</td>
<td>1.26</td>
<td>0.38</td>
<td>0.32</td>
<td>Trace</td>
</tr>
<tr>
<td>Valine</td>
<td>1.16</td>
<td>0.36</td>
<td>0.40</td>
<td>Trace</td>
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</table>

Amino acid analyzer J-EOL-GAH: Buffer Lithium citrate solution (pH 2.2).
Samples: A = Barangay Lioac, Naguilian, La Union; B = Barangay San Mateo, Laoag City; C = Barangay Cili, Binalonan, Pangasinan and D = Piddig, Ilocos Norte.
leucine (0.06 to 2.39), lysine (0.09 to 2.36), phenylalanine (0.31 to 5.0), proline (trace to 1.66), serine (0.05 to 0.99), threonine (0.06 to 1.12), tyrosine (trace to 1.26) and valine (trace to 1.16). Trace amounts of cystine and methionine were found on all the samples.

Generally, the La Union sample has higher amounts of free amino acids compared with the other samples except for glutamic acid which was highest in the sample from Barangay Cili, Binalonan, Pangasinan (2.63 mg/100 ml).

The amino acid content of fresh sugarcane juice has been studied by Wiggins (1953) based on the method of Worwod. The procedure was carried out in eleven strip chromatograms simultaneously on one large piece of filter paper, placing a known volume of amino acid concentrate at intervals along the top of the paper, developing the end strip with ninhydrin in order to locate the amino acids. Then horizontal strips were cut and so as to include in each the separate amino acid absorbed on the paper from ten chromatograms, thus obtaining sufficient material for reasonably accurate analysis. Each strip was eluted with hot dilute ammonia and the eluate analyzed for its amino acid content by Worwod’s procedure.

Results showed that the cane juice consisted of five major amino acids namely: aspartic (1.36 micromoles/100 ml), glutamic acid (1.2), alanine (1.65), valine together with gamma-aminobutyric (0.25) and leucine (0.98). Other amino acids found in small amounts were asparagine, serine, glycine and lysine.

Based on the analysis of cane juice there were five major and five minor acids. The amino acids found in the fresh cane juice were also present in the sugarcane wine except for the presence of asparagine and gamma-aminobutyric acid in cane juice but were found absent in sugarcane wine. On the other hand, sugarcane wine also consisted of arginine, cystine, histidine, isoleucine, methionine, phenylalanine, proline, threonine and tyrosine. These differences in results can be traced to the differences in the methods of analyses used, variety of sugarcane and the possible effect of fermentation on the cane juice. Conclusive results can be obtained on the action of microorganisms on cane juice if the same raw material can be subjected to amino acid analysis using Amino Acid Analyzer.

E. Polyphenol composition of additives.

Duhat and “samac” barks were added during the “basi” preparation to impart color and flavor and also control the bacterial contaminations. “Samac” leaves and fruit besides these functions, also served as source of inocula.

These additives were chemically analyzed using Thin Layer Chromatography following the method of Folin-Dennis. Results showed that duhat bark contains myricetin, (+) - epigallocatechin, (-) -epicatechin and theogallin (Fig. 4). “Samac” bark contains only (+) epigallocatechin gallate and some unidentified substances (Fig. 5).

On the other hand, “samac” leaves contained only myricetin and other unidentified substances while its fruits only had (−) -epicatechin and some unidentified substances (Figs. 6 and 7). However, all the additives contained (−) - catechin.

The polyphenol present differs in their molecular weights, optical rotation and melting point as follows (Devon, 1975):

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Figure 4. Thin layer chromatogram of polyphenols in duhat bark.

Figure 5. Thin layer chromatogram of polyphenols in “samac” bark.
Studies have shown that these polyphenols except for myricetin and theo-
gallin, were present also in green tea and “tangal” bark (Nishiyama and Kozaki, 1974 and 1975, Nishiyama et al., 1978). They were found to inhibit lactic acid bacteria.

Figure 6. Thin layer chromatogram of polyphenols in “samae” leaves.

Figure 7. Thin layer chromatogram of polyphenols in “samae” fruits.

**LITERATURE CITED**


NISHIYAMA, R., P.C. SANCHEZ and M. KOZAKI. 1978. Inhibitory function of mangrove bark toward all growth of micro-


