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RESEARCH ARTICLE

Investigation of Star Gooseberry (*Phyllanthus Acidus)* Wine Production

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Abstract

The star gooseberry fruit is pendulous, bear in small clusters from the branches, round or slightly flattened at the poles. Gooseberry fruit is rich in antioxidants including ascorbic acid and phenolic compounds. It is an important herbal medicine due to its effective antiviral activities. Effect of primary fermentation temperature (26^{o}C, 28^{o}C, 30^{o}C, 32^{o}C), sugar to pulp ratio (4, 8, 12, 16% w/w), *Saccharomyces cerevisiae* inoculation (1.0 x 10^8, 1.5 x 10^8, 2.0 x 10^8, 2.5 x 10^8 cfu/g) on ascorbic acid (mg/ 100 mL), total phenolic content (mg GAE/ 100 mL) and total flavonoid (mg CE/ 100 mL) content of *Phyllanthus acidus* wine were thoroughly investigated. The aging (1, 2, 3, 4 weeks) was also examined to improve the turbidity (mJ/cm^2) and sensory score of wine. The optimal conditions for fermentation of *Phyllanthus acidus* juice should be conducted at 12 days of primary fermentation with 12% of sugar supplementation inoculated with 2.0 x 10^8 cfu/ml of *Saccharomyces cerevisiae* and 4 weeks of aging to get pleasant physico-chemical characteristics of *Phyllanthus acidus* wine. *Phyllanthus acidus* wine was red-pink, clear and transparent. This present study revealed that horticulture farmers would get more added values from *Phyllanthus acidus* fruits not only by local commerce but also processing. It could be processed by fermentation and conversion into high valued wine.

Keywords: *Phyllanthus acidus, Wine, Fermentation, Sacchromyces cerevisiae, Ascorbic acid, Phenolic, flavonoid.*

Introduction

Gooseberry (*Phyllanthus acidus*) tree has been cultivated in Vietnam. *Phyllanthus acidus* belongings to genus *Phyllanthus* (Euphorbiaceae). Star gooseberry plants starts flowering 3-4 years after planting. The tree usually produces flowers and fruits twice a year and bear fruits heavily; each inflorescence bears 30-60 fruits, which takes 40-45 days to get matured. Fruits are green at first, but when they mature become pale yellow to nearly white when fully ripe. Fruits have good shelf-life and can be kept for 8-10 days without any deterioration in their quality under ambient temperature conditions [1].

Gooseberry fruit is rich in antioxidants including ascorbic acid and phenolic compounds [2]. This fruit is eaten fresh, candied in sugar or pickled in salt. In addition, gooseberry is also used to make fruit juice. It produces useful secondary metabolites such as alkaloids, tannins, flavanoids, lignans, phenolics and terpenes [3]. Fruits are borne in loose clusers, are pale yellow or white, waxy, crisp and juicy, and very sour. Several therapeutic properties including antiviral, antibacterial, neuroprotective, antifibrosis, and anticancer activities have also been reported for *Phyllanthus acidus* [4, 7].

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Phyllanthus acidus leaf extract exert hypoglycemic activity in diabetic by stimulating insulin secretion [8]. *P. acidus* crude water extract also strongly expressed the capacity to retard lipid oxidation, radical scavenging, radical cation decolorization and reducing power in minced pork. *P. acidus* leaf extract could be used as natural antioxidant in the pork industry [9]. Fermentation is a relatively efficient, low energy preservation process which increases the shelf life, and decreases the need for refrigeration or other forms of food preservation technology.

Fruit wines are undistilled alcoholic beverages usually made from fruits which are nutritive, more tasty, and mild stimulants. Fruits undergo a period of fermentation and aging. They usually have an alcohol content ranging between 5 and 13%. Wine is a food with a flavor like fresh fruit which could be stored and transported under the existing conditions. Being fruit-based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice.

The nutritive value of wine is increased due to the release of amino acids and other nutrients from yeast during fermentation. Fruit wines contain 8–11% alcohol and 2–3% sugar with energy value ranging between 70 and 90 kcal per 100 ml [10]. There were some studies mentioned to *Phyllanthus acidus* wine fermentation. Experiment was to produce wine from star gooseberry (*Phyllanthus acidus* (L) Skeels and carambola (*Averrhoa carambola* L.) by fermented with *Saccharomyces cerevisiae* for two weeks [11]. Production of wine from Indian star gooseberry was investigated [12]. There is an abundance of *Phyllanthus acidus* fruits in harvesting season with the potential to be used for wine fermentation. Being fruit-based fermented and undistilled product, *Phyllanthus acidus* wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to the release of amino acids and other nutrients from yeast during fermentation.

Material & Method

Material

Approximately 8 kg of *Phyllanthus acidus* fruits was collected in Hau Giang province, Vietnam. The selected fruits were all at commercial maturity level, without damage, insect and foreign matter. Sugar was purchased from local market. All chemicals used were analytical grade. They were washed with tap water to remove dirt and residues followed by air dried.

Fermentation of *Phyllanthus acidus* was carried out in 5 L stainless steel fermentation tanks. *Phyllanthus acidus* fruits were blended and poured into fermentation tank and added with sugar in alternate layer. Then, fermentation tank was closed tightly and stored at different temperature. The wine from each tank was strained, pasteurized at 58°C for 16 s and stored in bottles at -18°C until further analysis.

Figure 1: ***Phyllanthus acidus***

Research Method

Effect of Primary Fermentation Temperature on Ascorbic Acid, Total Phenolic Content and Total flavonoid Content As well As Physicochemical Properties of Phyllanthus Acidus Wine

Four levels of primary fermentation temperature (°C) namely (i) 26°C (ii) 28°C (iii) 30°C (iv) 32 °C using 4% white sugar to pulp ratio (w/w) and 1.0 x 10^8 cfu/g of *Saccharomyces cerevisiae* inoculation were carried out. The best primary fermentation duration was selected based on the values of ascorbic acid (mg/ 100mL), total phenolic (mg GAE/ 100mL), total flavonoid (mg CE/ 100mL); physicochemical properties such as ethanol (%), pH, total soluble solid (°Brix) at day 12th of *Phyllanthus acidus* wine.

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Effect of White Sugar to Pulp Ratio on Ascorbic Acid, Total Phenolic Content and Total flavonoid Content as Well as Physicochemical Properties of Phyllanthus Acidus Wine

Four levels of white sugar to pulp ratio namely (i) 4% (ii) 8% (iii) 12% (iv) 16% (w/w) were used in fermentation. The optimum fermentation temperature determined from previous section was chosen (30^{o}C). All samples were used 1.0 x 10^8 cfu/g of *Saccharomyces cerevisiae* inoculation for fermentation.

The best white sugar to pulp ratio was selected based on the values of ascorbic acid (mg/ 100mL), total phenolic (mg GAE/ 100mL), total flavonoid (mg CE/ 100mL); physicochemical properties such as ethanol (%), pH, total soluble solid (^{o}Brix) at day 12th of *Phyllanthus acidus* wine..

Effect of Yeast Saccharomyces Cerevisiae Inoculation on Ascorbic Acid, Total Phenolic Content and Total flavonoid Content as Well as Physicochemical Properties of Phyllanthus Acidus Wine

Four levels of yeast *Saccharomyces cerevisiae* inoculation (cfu/ml) namely (i) 1.0 x 10^8 (ii) 1.5 x 10^8 (iii) 2.0 x 10^8 (IV) 2.5 x 10^8 (w/w) were used in fermentation. The optimum primary fermentation temperature determined from previous section was chosen (30^{o}C). The optimal white sugar to pulp ratio was chosen (12%).

The best yeast *Saccharomyces cerevisiae* inoculation (cfu/ml) was selected based on was selected based on the values of ascorbic acid (mg/ 100mL), total phenolic (mg GAE/ 100mL) and total flavonoid (mg CE/ 100mL); physicochemical properties such as ethanol (%), pH, total soluble solid (^{o}Brix) of at day 12th of *Phyllanthus acidus* wine.

Effect of Secondary Fermentation Duration (aging) on Turbidy and Sensory Properties of Phyllanthus Acidus Wine

Four levels of secondary fermentation duration namely (i) 1 week (ii) 2 weeks (iii) 3 weeks (iv) 4 weeks were carried out at 9^{o}C. The best secondary fermentation duration was selected based on the values of turbidity (mJ/cm^2) and sensory score (9-point hedonic scale) of *Phyllanthus acidus* wine.

Physico-chemical and Statistical Analysis

The ethanol content of sample was determined using gas chromatography. The pH of samples was measured using pH meter. Hand-held refractometer with detection Brix° range of 0-32 Brix° was used to determine the total soluble solid of samples. Turbidity (mJ/cm^2) was measured by spectrophotometer. Sensory score was based on 9-point hedonic scale. The total phenolic contents were determined with the

Folin-Ciocalteu assay [13]. Total flavonoid content was determined with the aluminum chloride colorimetric assay, using catechin as a standard [14]. Spectrophotometric method for determination of L-ascorbic acid in fruit juices was used [15]. Data were statistically summarized by Statgraphics Centurion XVI.

Result & Discussion

Effect of Primary Fermentation Temperature on Ascorbic Acid, Total Phenolic Content and Total flavonoid Content of *Phyllanthus Acidus* Wine

A study was to evaluate antioxidant, cytotoxic and antimicrobial activities of methanolic extracts of pulp and seed of *Phyllanthus acidus*. Maximum phenolic (25.672± 0.645 mg gallic acid equivalents/mg of plant extract) and flavonoid (13.893 ± 0.320 mg catechin equivalents/mg of plant extract) contents were found in pulp extract than seed extract.

Both the pulp and seed extracts showed the potent antioxidant activity with IC50 value of 5.96 µg and 6.79 µg/mL respectively which are very close to the IC50 value of standard ascorbic acid having 2.16 µg/mL) [16]. During fermentation, there are several factors that winemakers take into consideration. The most notable is that of the internal temperature of the must. The biochemical process of fermentation itself creates a lot of residual heat which can take the must out of the ideal temperature range for the wine [17].

A lower temperature is desirable because it increases the production of esters, other aromatic compounds, and alcohol itself. This makes the wine easier to clear and less susceptible to bacterial infection [18]. Temperature control during alcoholic fermentation is necessary to facilitate yeast growth, extract flavors and colors from the

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skins, permit accumulation of desirable by-products, and prevent undue rise in temperature that might kill the yeast cells. The low temperature and slow fermentation favor the retention of volatile compound [19].During fermentation; yeast interacts with sugars in the juice to create ethanol, commonly known as ethyl alcohol and carbon dioxide as a by-product.

In winemaking, the temperature and speed of fermentation are an important consideration as well as the levels of oxygen present in the must at the start of the fermentation [10]. Experiment was to produce wine from star gooseberry (*Phyllanthus acidus* (L) Skeels and carambola (*Averrhoa carambola* L.) by fermented with *Saccharomyces cerevisiae* for two weeks. Results showed that star gooseberry wine gave significantly higher total acid (%TA) than carambola wine at all formulations but the star gooseberry wine had lower acidity than carambola wine. Star gooseberry wine gave significantly higher in ethyl alcohol production (averaged 15.90%) than carambola wine (averaged 8.28%).

Meanwhile, star gooseberry wine formulation 4 gave the highest ethyl alcohol (23.12%), and followed by carambola wine formulation 4 (14.37%), star gooseberry wine formulation 3 (17.25%), star gooseberry wine formulation 2 (13.75%), star gooseberry wine formulation 1 (9.5%), carambola wine formulation 3 (8.75%), carambola wine formulation 2 (6.5%) and the lowest ethyl alcohol production in carambola wine formulation 1 (3.5%). The amount of ethyl alcohol was analyzed in each formulation both in star gooseberry wine and carambola wine [11].

Table 1: Effect of primary fermentation temperature on ascorbic acid, total phenolic content and total flavonoid content of *Phyllanthus acidus* wine

Fermentation temperature (°C)	Ascorbic acid (mg/ 100mL)	Total phenolic (mg GAE/ 100mL)	Total flavonoid (mg CE/ 100mL)	Ethanol (%)	pH	TSS (°Brix)
26	24.19±0.01^{c}	224.32±0.01^{b}	25.78±0.02^{c}	1.45±0.01^{b}	3.57±0.01^{a}	22.25±0.01^{a}
28	25.08±0.00^{b}	228.53±0.01^{ab}	26.21±0.01^{ab}	2.71±0.02^{ab}	3.56±0.02^{ab}	20.01±0.01^{b}
30	**26.17±0.01^{a}**	**241.50±0.00^{a}**	**26.31±0.01^{a}**	**3.47±0.01^{a}**	**3.55±0.01^{b}**	**17.30±0.01^{c}**
32	24.99±0.02^{bc}	201.10±0.03^{c}	26.01±0.01^{b}	4.12±0.01^{a}	3.55±0.03^{b}	16.17±0.02^{d}

Data are expressed as mean ± SD values (n = 3). Mean value with different superscript in each column differs signifcantly (p<0.05)

From table 1, the primary fermentation should be carried out at the 12th day to achieve the optimal ascorbic acid, total phenolic content and total flavonoid content of *Phyllanthus acidus* wine.

Effect of White Sugar to Pulp Ratio on Ascorbic Acid, Total Phenolic Content and Total flavonoid Content as Well as Physicochemical Properties of *Phyllanthus Acidus* Wine

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, and hydrogen [20, 21]. Sugar may need to be added to spur the fermentation process in the event that the fruit does not contain enough natural sugar to ferment on its own in the presence of yeast. Sucrose and water can be added to help counter the fruit's tart acidity.

Table 2: Effect of sugar to pulp ratio on ascorbic acid, total phenolic, total flavonoid content; physicochemical properties of *Phyllanthus acidus* wine at day 12th

Sugar: pulp (% w/w)	Ascorbic acid (mg/100mL)	Total phenolic (mg GAE/ 100mL)	Total flavonoid (mg CE/ 100mL)	Ethanol (%)	pH	TSS (°Brix)
4	26.17±0.01^{ab}	241.50±0.00^{ab}	26.31±0.01^{ab}	3.47±0.01^{b}	3.55±0.01^{a}	17.30±0.01^{c}
8	26.23±0.02^{ab}	263.48±0.02^{ab}	26.49±0.02^{ab}	3.59±0.02^{ab}	3.55±0.00^{a}	18.52±0.02^{b}
12	**27.04±0.01^{a}**	**279.47±0.01^{a}**	**26.94±0.01^{a}**	**3.77±0.01^{a}**	**3.54±0.02^{a}**	**19.73±0.01^{ab}**
16	25.93±0.01^{b}	236.29±0.01^{b}	26.22±0.01^{b}	3.80±0.00^{a}	3.54±0.00^{a}	20.31±0.02^{a}

Data are expressed as mean ± SD values (n = 3). Mean value with different superscript in each column differs signifcantly (p<0.05)

Although sugar is an important substrate of fermentation, higher sugar concentration inhibits the growth of microorganisms [22]. From table 2, the optimal sugar to pulp ratio on ascorbic acid, total phenolic, total flavonoid content; physicochemical properties of *Phyllanthus acidus* wine should be performed at 12% w/w. Production of wine from Indian star gooseberry was investigated. PH show a decreased trend then attains minima and then increased. As the fermentation days proceed, the specific gravity increased and the alcohol percentage increased gradually [12].

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Effect of Yeast *Saccharomyces Cerevisiae* Inoculation on Ascorbic Acid, Total Phenolic Content and Total flavonoid Content as Well as Physicochemical Properties of *Phyllanthus Acidus* Wine

Molds and yeasts are usually low pH tolerant and are therefore associated with the spoilage of foods with low pH. Yeasts can grow in a pH range of 4–4.5 and molds can grow from pH 2–8.5 but favor low pH [23].During fermentation, the pH of the wine reaches a value of 3.5–3.8, suggesting that an acidic fermentation takes place at the same time as the alcoholic fermentation. Final alcohol content was about 7–8% within a fortnight [24].

The products of fermentation are as follows: Alcohol, glycerol, and carbon dioxide are obtained from yeast fermentation. Since yeasts function best in slightly acid medium, the mash, juice, sap, or extract prepared for fermentation must be checked for adequate acidity. If acidity is insufficient, acid or acid-bearing materials are added.

Using species of *S. cerevisiae* which converts the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters, and other chemical compounds which also help to preserve the wine [25, 27].

Table 3: Effect of yeast *Saccharomyces cerevisiae* inoculation (cfu/ml) on ascorbic acid, total phenolic, and total flavonoid content; physicochemical properties of *Phyllanthus acidus* wine at day 12^{th}

Saccharomyces cerevisiae count (cfu/ ml)	Ascorbic acid (mg/100mL)	Total phenolic (mg GAE/ 100mL)	Total flavonoid (mg CE/ 100mL)	Ethanol (%)	pH	TSS (oBrix)
1.0 x 108	27.04±0.01b	279.47±0.01ab	26.94±0.01b	3.77±0.01b	3.54±0.02a	19.73±0.01a
1.5 x 108	27.43±0.02ab	282.17±0.01ab	27.19±0.01ab	3.92±0.01ab	3.53±0.01a	19.42±0.00ab
2.0 x 108	**28.19±0.01a**	**291.04±0.02a**	**27.75±0.02a**	**4.37±0.03a**	**3.52±0.00a**	**19.04±0.01b**
2.5 x 108	27.15±0.01b	274.13±0.00b	26.58±0.01b	4.40±0.01a	3.52±0.02a	18.30±0.01c

Data are expressed as mean ± SD values (n = 3). Mean value with different superscript in each column differs signifcantly (p<0.05)

From table 3, the fermentation process should be conducted with 2.0 x 10^8 (cfu/ml) of *Saccharomyces cerevisiae* inoculation to achieve the most ascorbic acid, total phenolic, total flavonoid content; physicochemical properties of *Phyllanthus acidus* wine.

Effect of secondary fermentation duration (aging) on turbidity and sensory properties of *Phyllanthus acidus* wine

After fermentation was completed, the wine is separated from the sediment by racking. It can also be clarified further using fining agents such as gelatin, pectin, or casein which are mixed with the wine. Filtration can be carried out with filter aids such as fuller's earth after racking. Clarification of juice for white wine is usually achieved by aging, sedimentation or centrifugation. During aging and subsequent maturing in bottles, many reactions, including oxidation, occur with the formation of traces of esters and aldehydes, which together with the tannin and acids already present to enhance the taste, aroma, and preservative properties of the wine [28].The pH plays an important role in aging, clarifying, or fining. As the strength of the relative charge of suspended particles decreases in the wine, the pH of the wine increases.

At high pH, organic protein fining agents may possess a positive charge insufficient to bind to the negatively charged particulates, thus potentially increasing the turbidity of the wine [29]. Acids present in wine enhance the taste, aroma, and preservative properties of the wine. As soon as the desired degree of sugar disappearance and alcohol production has been obtained, the microbiological phase of winemaking is over [30].The wine was then pasteurized at 50°C–60°C. The temperature should be controlled so as not to heat it to about 70°C, since its alcohol content would vaporize at a temperature of 75°C–78°C [31].

Table 4: Effect of aging on turbidity and sensory properties of *Phyllanthus acidus* wine

Duration of aging (weeks)	Turbidity (mJ/cm^2)	Sensory score
1	57.23±0.01^{a}	7.21±0.01^{b}
2	55.36±0.01^{ab}	7.53±0.01^{ab}
3	54.19±0.02^{ab}	7.61±0.03^{ab}
4	52.37±0.01^{b}	8.09±0.02^{a}

Data are expressed as mean ± SD values (n = 3). Mean value with different superscript in each column differs signifcantly (p<0.05)

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From table 4, the secondary fermentation (aging) in 4 weeks at 9°C was necessary to get pleasant physico-chemical characteristics of *Phyllanthus acidus* wine.

Conclusion

Star gooseberry fruits are borne in loose clusters, are pale yellow or white, waxy, crisp and juicy, and very sour, found. It contains high contents of vitamin C to be used for used for improving eyesight and memory and preventive action against diabetes. The nutritive value of wine is increased due to the release of amino acids and other nutrients from yeast during fermentation. Wine could be prepared from nutritionally diverse, highly perishable, underutilized tropical, subtropical, or temperate fruits, thereby helping efforts to increase shelf life by reducing post-harvest and production losses, improve nutritional value of fruits, increase consumption and export, increase cultivation and commercialization of fruits as well as to generate profits to growers.

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