ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Several Parameters Influencing Wine Production from Mangrove Apple (*Sonneratia ovata)* Fruit

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Abstract

The ripe mangrove apple is a potential source of natural antioxidants owing to its significant antioxidant activities. Phytochemical analysis of fruit extract revealed the presence of carbohydrate, protein, flavonoid and phenolic compounds. It can enhance the capability of antioxidant human body and therefore reduce the risk of chronic diseases. It is sweet in taste and usually consumed fresh or made into juice. There is limited study mentioning to processing of this functional fruit. Therefore we explored a wine fermentation from mangrove apple *(Sonneratia ovata)* fruit by focusing on the effect of different variables such as sugar supplementation, innoculum size, fermentation temperature in the primary fermentation, and fermentation time and temperature in the maturation to mangrove apple *(Sonneratia ovata)* wine quality (%v/v alcohol; g/l titratable acidity; ^{o}Brix residual sugar; sensory score). Our results proved that the primary fermentation should be conducted with 12% sugar ratio, 0.25% *Sacchromyces cerevisiae* inoculum at temperature 30.0^{o}C in 15 days. The maturation was adequate in 10.0^{o}C for 3 weeks to get a pleasant flavor and aroma. Mangrove apple *(Sonneratia ovata)* wine also evaluated the antioxidant capacity and total phenolic content. Mangrove apple *(Sonneratia ovata)* wine could be considered as a healthy source of antioxidants and phenolics ideal for daily consumption.

Keywords: *Mangrove apple (Sonneratia ovata), Wine, fermentation, Sacchromyces cerevisiae, Antioxidant, Phenolic.*

Introduction

Mangrove apple (*Sonneratia ovata*) have naturally grown in riverside Soc Trang province, Viet Nam. Sonneratia is a typical mangrove genus comprising of nine species [1]. It is popularly widespread in tropical tideland. *Sonneratia ovata* is a small tree with oblong or obovate-elliptic coriaceous leaves and large [2]. *S. ovata* can be distinguished by several attributes including the presence of a finely warted texture on the calyx surface forming a cup enclosing the base of the fruit, fruit apex that is depressed at the base of the style [3].

Berry globose, pericarp leathery, apex of fruit depressed at base of style [4]. Extracts of this plant are traditionally used as an astringent and antiseptic. It contains alkaloid, tanin, flavonoid, saponin, phytosterol, and carbohydrate [5, 6]. It exhibits antimicrobial activities against certain microorganisms [7,8].

The existence of most of the phytochemicals in the leaves showed some important biological activities [9].

It has hypoglycemic effect as dietary fiber [10], antioxidant and anticholinesterase activities [11]. *Sonneratia* plants have been used in traditional medicine to treat diseases such as asthma, fever, ulcers, hepatitis, hemorrhoids (piles), sprain and hemorrhages [12]. The half ripe fruits are used to relieve cough, the ripe fruits are used as anthelmintic drug and the fermented fruit juice is said to be useful in arresting haemorrhage [11].

They have traditionally been used for the treatment of hemorrhage, swelling, intestinal parasites and coughs [13, 14]. The apple mangrove extract could be employed in shrimp culture as a prophylactic/ therapeutant as well as an immunostimulant without negative effects on growth, nutrient utilization and carbohydrate and protein digestion [15]. S. *ovata* has shown strong antioxidant activity and anti-lipid peroxidation, intestinal α-glucosidase inhibitory activity, potentiation of pancreatic secretion of insulin, high glucose uptake from

serum and low glucose absorption from gut [16, 20].In order to utilize mangrove apple (*Sonneratia ovata*) as a healthy food drink, Nguyen Phuoc Minh [21] attempted to produce mangrove apple juice. Mangrove apple *(Sonneratia ovata)* is an underutilized fruit crop and still now there is very limited research available regarding to processing of this fruit into value added product.

Fermentation is a relatively efficient, low-energy preservation process which increases the shelf life and decreases the need for refrigeration or other form of food preservation technology [22]. Therefore, we utilized this fruit as subtrate for wine fermentation. We focused on the effect of different technical variables such as sugar ratio, innoculum size, fermentation temperature in the primary fermentation, and fermentation time and temperature in the maturation to mangrove apple *(Sonneratia ovata)* wine quality.

Material & Method

Material

Ripen mangrove apple *(Sonneratia ovata)* fruits were naturally collected from Soc Trang province, Vietnam. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Their pulps were utilized for wine fermentation. Apart from collecting mangrove apple *(Sonneratia ovata)*, we also used other materials such as sugar, yeast (*Saccharomyces cerevisae*). Lab utensils and equipments included weight balance, refractometer, pH meter, ethanol meter, thermometer, water bath, buret, erlenmeyer flask, glassware.

Figure 1: ***Sonneratia ovata***

Research Method

Effect of Sugar Supplementation in the Primary Fermentation

Mangrove apple *(Sonneratia ovata)* pulp was diluted with water (1:3) to form a condensed solution. Then this solution will be mixed with sugar in different sugar ratio (4 %, 8 %, 12%, 16 %). The primary fermentation was conducted at ambient temperature (28 ^{o}C) in 15 days with 0.15% *Saccharomyces cerevisiae*. At the 15th day of fermentation we took the must to analyze alcohol content (% v/v), acidity (g/l), residual sugar (^{o}Brix), sensory score.

Effect of Innoculum Size in the Primary Fermentation

Mangrove apple *(Sonneratia ovata)* pulp was diluted with water (1:3) to form a condensed solution. Then this solution will be mixed with sugar with sugar (12%). The primary fermentation was conducted at ambient temperature (28^{o}C) in 15 days with *Saccharomyces cerevisiae* at different ratio (0.15%, 0.20%, 0.25%, 0.30%).At the 15th day of fermentation we took the must to analyze alcohol content (% v/v), acidity (g/l), residual sugar (^{o}Brix), sensory score.

Effect of Fermentation Temperature in the Primary Fermentation

Mangrove apple *(Sonneratia ovata)* pulp was diluted with water (1:3) to form a condensed solution. Then this solution will be mixed with sugar with sugar (12%).

The primary fermentation was conducted at ambient temperature (28°C) in 15 days with *Saccharomyces cerevisiae* (0.25%) at different temperature (28.0°C, 29.0°C, 30.0°C, 31.0°C) by setting erlenmeyer flasks in water bath in 15 days. At the 15th day of fermentation we took the must to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), sensory score.

Effect of Fermentation Time and Temperature in the Maturation

After completing the primary fermentation of mangrove apple *(Sonneratia ovata)* fruits with supplementation of sugar (12%), *Saccharomyces cerevisiae* (0.25%), fermentation temperature (30.0°C) in 15 days; the must should come to the maturation step by keeping mangrove apple *(Sonneratia ovata)* must at different temperature (9.0°C, 10.0°C, 11.0°C, 12.0°C) by different time (1, 2, 3, 4 weeks) to improve the mangrove apple *(Sonneratia ovata)* wine quality. Weekly, mangrove apple *(Sonneratia ovata)* wine would be monitored the sensory score.

Antioxidant Capacity and Total Phenolics Contents in Mangrove Apple (Sonneratia ovata) Wine

Mangrove apple *(Sonneratia ovata)* wine quality was aslo evaluated on antioxidant capacity and total phenolic content. Antioxidant capacities were determined by DPPH (μM TE/g) and FRAP (μM TE/g). Total phenolics content was also examined by TPC (mg GAE/g).

Physico-chemical and Sensory Measurement

Alcohol content (%v/v) was analyzed by gas chromatography [23]. Acidity (g/l) was determined by using 5 ml of sample and titrated with 0.1N NaOH with phenolphthalein as indicator [24]. Residual sugar (°Brix) was analyzed by refractometer. Sensory score was performed by panelist of 12 members based on the Hedonic 9 points. DPPH assay followed according to Rufino *et al.* [25]. The analysis of FRAP was performed according to Thaipong *et al.*

Was determined using the Folin-Ciocalteu reagent [26].

Statistical Analysis

Data were statistically summarized by Statgraphics Centurion XVI.

Result & Discussion

Effect of Sugar Supplementation in the Primary Fermentation

Fermented fruit wines are popular throughout the world, and in some regions, it makes a significant contribution to the diet of millions of individuals [22]. The process of fermenting is basically feeding sugars and nutrients in solution to yeast, which return the favor by producing carbon dioxide gas and alcohol [27]. Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, and hydrogen [28].

Mangrove apple *(Sonneratia ovata)* pulp was diluted with water (1:3) to form a condensed solution. Then this solution will be mixed with sugar in different sugar ratio (4%, 8%, 12%, 16%).The primary fermentation was conducted at ambient temperature (28°C) in 15 days with 0.15% *Saccharomyces cerevisiae*. At the 15th day of fermentation we took the must to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), sensory score. Results were clearly presented in table 1. It's obviously noted that 12% sugar supplementation was optimal for mangrove apple *(Sonneratia ovata)* must.

Table 1: Effect of sugar supplementation (%) to alcohol content (% v/v), titratable acidity (g/l), residual sugar (°Brix), sensory score of mangrove apple *(Sonneratia ovata)* must

Sugar supplementation (%)	4	8	12	16
Alcohol content (% v/v)	2.07±0.05c	3.18±0.03ab	3.96±0.03a	2.51±0.02b
Titratable acidity (g/l)	2.19±0.02b	2.46±0.02ab	2.61±0.02a	2.31±0.02ab
Residual sugar (oBrix),	0.91±0.06d	2.09±0.07c	3.14±0.03b	6.05±0.04a
Sensory score	5.07±0.04c	6.51±0.02b	7.40±0.05a	5.83±0.01bc
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)				

Although sugar is an important substrate of fermentation, higher sugar concentration inhibits the growth of microorganisms [29]. 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 [22].

Effect of Innoculum Size in the Primary Fermentation

As a living organism, yeast primarily requires sugars, water, and warmth to stay alive. Albumen or nitrogenous material is also necessary for yeast to thrive [22]. The size of the inocula is a well known key process parameter in microbial fermentation [30, 31]. Some of these studies showed that a higher level of inoculum resulted in higher fermentation rates [32]. Higher inoculum size resulted in higher yields of glycerol and ethyl alcohol [33].

Mangrove apple *(Sonneratia ovata)* pulp was diluted with water (1:3) to form a condensed solution. Then this solution will be mixed with sugar with sugar (12%). The primary fermentation was conducted at ambient temperature (28^{o}C) in 15 days with *Saccharomyces cerevisiae* at different ratio (0.15%, 0.20%, 0.25%, 0.30%).At the 15th day of fermentation we took the must to analyze alcohol content (% v/v), acidity (g/l), residual sugar (^{o}Brix), sensory score. Results were comprehensively noted in Table 2.

Table 2: Effect of yeast ratio (%) to alcohol content (% v/v), titratable acidity (g/l), residual sugar (^{o}Brix), sensory score of mangrove apple *(Sonneratia ovata)* must

Yeast ratio (%)	0.15	0.20	0.25	0.30
Alcohol content (% v/v)	3.96±0.03^{a}	4.35±0.05^{ab}	4.96±0.03^{a}	5.05±0.02^{a}
Titratable acidity (g/l)	2.61±0.02^{b}	2.68±0.01^{ab}	2.73±0.04^{ab}	2.75±0.04^{a}
Residual sugar (^{o}Brix),	3.14±0.03^{a}	2.71±0.04^{ab}	2.32±0.03^{b}	2.28±0.05^{b}
Sensory score	7.40±0.05^{b}	7.68±0.06^{ab}	7.87±0.05^{a}	7.90±0.01^{a}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)				

Higher alcohols, iso-acids and fatty acids in general are not desirable compounds from the sensory point of view of wine over certain concentration levels [34]. there are no significant differences between size of inoculum and aroma compounds concentration, except for higher alcohols and ethyl acetate [35, 36]. The influence of pitching rate on aroma compound production is rather limited, with the exception of total diacetyl levels, which strongly increase with inoculum size [37, 38].

A consistent increase of desired aroma compounds (esters, lactones and free monoterpenes), and a decrease of less desired compounds (higher alcohols and medium chain fatty acids), was shown at inoculum sizes of 105 cells/mL for both strains in real winemaking conditions [32].

Effect of Fermentation Temperature in the Primary Fermentation

During fermentation, 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 [39]. Juice temperature must be warm for fermentation. However, yeast cells will die if temperature is too hot [40]. Mangrove apple *(Sonneratia ovata)* pulp was diluted with water (1:3) to form a condensed solution. Then this solution will be mixed with sugar with sugar (12%).

The primary fermentation was conducted at ambient temperature (28^{o}C) in 15 days with *Saccharomyces cerevisiae* (0.25%) at different temperature (28.0^{o}C, 29.0^{o}C, 30.0^{o}C, 31.0^{o}C) by setting erlenmeyer flasks in water bath in 15 days. At the 15th day of fermentation we took the must to analyze alcohol content (% v/v), acidity (g/l), residual sugar (^{o}Brix), sensory score. Results were depicted in table 3. The optimal temperature was indicated at 30^{o}C.

Table 3: Effect of fermentation temperature (^{o}C) to alcohol content (% v/v), titratable acidity (g/l), residual sugar (^{o}Brix), sensory score of mangrove apple *(Sonneratia ovata)* must

Fermentation temperature (^{o}C)	28.0	29.0	30.0	31.0
Alcohol content (% v/v)	4.96±0.03^{b}	5.11±0.03^{ab}	5.64±0.05^{a}	5.25±0.01^{ab}
Titratable acidity (g/l)	2.73±0.04^{b}	2.80±0.02^{ab}	2.89±0.03^{a}	2.82±0.04^{ab}
Residual sugar (^{o}Brix),	2.32±0.03^{a}	2.03±0.04^{b}	1.82±0.02^{c}	1.91±0.03^{bc}
Sensory score	7.87±0.05^{b}	7.95±0.01^{ab}	8.06±0.04^{a}	8.00±0.02^{ab}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)				

Temperature control during alcoholic fermentation is necessary to facilitate yeast growth, extract flavors and colors from the 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 compounds [41].

Effect of Fermentation Time and Temperature in the Maturation

The wine maturation is related to steps of wine storage before bottling. During wine aging many reactions occurred that caused significant organoleptical changes in wines. During the process of maturation and aging, the most obvious change occurs in the color of the wine which refers to phenols changes [42].

The changes occurring in the phenols in wines during and after the production are realized by different mechanisms. In the aging of wines, it is intended to make a specific change in the composition of the wine by changing the organoleptic properties of the wine [43]. Temperature can play a significant role in the development of wine at many stages during its lifetime. Elevated temperature poses a significant risk to the sensory attributes of wine and its resultant shelf-life. If temperature is not adequately controlled across these critical control points, there is a significant risk to the physical, chemical and sensory attributes that the wine can portray [44].

After completing the primary fermentation of mangrove apple *(Sonneratia ovata)* fruits with supplementation of sugar (12%), *Saccharomyces cerevisiae* (0.25%), fermentation temperature (30.0^{o}C) in 15 days; the must should come to the maturation step by keeping mangrove apple *(Sonneratia ovata)* must at different temperature (9.0^{o}C, 10.0^{o}C, 11.0^{o}C, 12.0^{o}C) by different time (1, 2, 3, 4 weeks) to improve the mangrove apple *(Sonneratia ovata)* wine quality. Weekly, mangrove apple *(Sonneratia ovata)* wine would be monitored the sensory score. Results were elaborated in Table 4. Maturation in 3 weeks was noted in appropriated manner.

Table 4: Effect of maturation to sensory score of mangrove apple *(Sonneratia ovata)* wine

	Maturation at temperature (^{o}C)			
Maturation time (week)	9.0	10.0	11.0	12.0
1	8.13±0.02^{b}	8.28±0.01^{a}	8.06±0.04^{bc}	8.01±0.05^{c}
2	8.14±0.01^{b}	8.30±0.05^{a}	8.09±0.02^{bc}	8.05±0.02^{c}
3	8.17±0.04^{b}	8.31±0.02^{a}	8.11±0.02^{bc}	8.08±0.02^{c}
4	8.17±0.02^{b}	8.32±0.04^{a}	8.12±0.02^{bc}	8.08±0.02^{c}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)				

During storage, the volatile composition of wine changes because of different reactions taking place, in particular ester hydrolysis/esterification reactions. Changes in ester composition will occur during storage and the reaction rate is influenced by the storage temperature [44].

Wines that were stored at chilled conditions (0, 5 or 10°C) were shown to have prolonged life, retaining their youthful wine aromas [45, 46]. 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 [22].

Antioxidant Capacity and Total Phenolics Contents in Mangrove Apple *(Sonneratia ovata)* Wine

Flavour and aroma are arguably the most important contributors to perceived wine quality. The aroma of wine is influenced by a range of volatile compounds. Volatile compounds present in wine considered to be the most important to flavour are those arising from the fermentation process, which include ethyl esters, acetate esters, higher alcohols, fatty acids and aldehydes [47].

While volatile compounds are important for aroma perception in wine matrices, non-volatile compounds play an important role in the palate characteristics of a wine and, in

many cases; these can be equally sensitive to the impact of temperature. There are a range of important classes of non-volatile compounds typically found in wines (organic acids, polysaccharides, sugars and proteins), and the group of compounds most sensitive to the impacts of heat exposure is the phenolic substances. The chemical profile of wine can change significantly with elevated storage temperature, indicating that the reaction mechanisms and Arrhenius activation energies involved are highly sensitive to temperature [44]. Mangrove apple *(Sonneratia ovata)* wine quality was aslo evaluated on antioxidant capacity and total phenolics contents. Antioxidant capacity was determined by DPPH (µM TE/g) and FRAPS (µM TE/g). Total phenolics content was also examined by TPC (mg GAE/g). Results were expressed in table 5. From this result, the mangrove apple *(Sonneratia ovata)* wine had high phenolic content and antioxidant capacity which were ideal for daily consumption as a healthy food drink.

Table 5: Antioxidant capacity and total phenolic content in mangrove apple *(Sonneratia ovata)* wine

Parameter	DPPH (µM TE/g)	FRAP (µM TE/g)	TPC (mg GAE/g)
Value	369.47±0.83	31.07±0.29	77.65±0.15

Note: the values were expressed as the mean of three repetitions

Khumaidah Laili et al [48].Showed the highest in vitro antioxidant activity with IC50 values of 4.73 µg/ mL and 2.00µg/mL for DPPH and ABTS free radical scavenging respectively in *Sonneratia ovata* Backer extract**.** Many of the polyphenols and other bioactive compounds in the source materials are bonded to insoluble plant compounds. The winemaking process releases many of these bioactive components into aqueous ethanolic solution, thus making them more biologically available for absorption during consumption [49].

Conclusion

Mangrove apple (*Sonneratia ovata*) is widely grown in riverside of tropical region. Its sour tasting young berry fruits are edible and applied as medicine in poultices to relieve sprain. It is quite a popular fruit usually consumed fresh or made into fruit juice or juice beverage. In order to utilize this good source as a healthy food drink, we attempted to produce mangrove apple wine.

We have successfully utilized mangrove apple *(Sonneratia ovata)* fruits as substrate for wine fermentation by investigating different parameters such as sugar ratio, innoculum size, fermentation temperature in the primary fermentation, and fermentation time and temperature in the maturation to mangrove apple *(Sonneratia ovata)* wine quality.These results were very important to diversify its processed products as well as enhance its added value.

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