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Some Variables Affecting To Gooseberry (*Phyllanthus acidus*) Wine Fermentation

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Abstract.

Wine is an alcoholic beverage typically made of fermented grape juice or variety of fruits. However, the natural balance of grapes is such that they can ferment without addition of sugars, acids, enzymes or other nutrients.Wine is produced by fermenting crushed fruits using various types of yeast. Gooseberry is an edible small yellow berries fruit in the Phyllanthus family. Fruits are borne in loose clusers, are pale yellow or white, waxy, crisp and juicy, and very sour. Its extract could be used as a potential therapeutics in many pathological conditions. There is limited study mentioning to processing of this nutritional fruit. Therefore we explored a wine fermentation from Gooseberry by focusing on the effect of different parameters such as pectinase concentration and time of treatment for juice extraction, yeast inculate for wine fermentation, and secondary fermentation to wine quality**.** Our results proved that 2.0% pectinase was used for juice extraction in 40 minutes, 1.5% *sacchromyces cerevisiae* was used for the main fermentation at 28^{o}C in 10 days, and 3 weeks of aging in dark bottle at 9.5^{o}C was applied to get a pleasant gooseberry quality. Using gooseberry having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely.

Keywords: *Gooseberry, wine, pectinase, fermentation, sacchromyces cerevisiae, aging*

1. INTRODUCTION

Phyllanthus acidus (L.) commonly known as gooseberry or amla, is a widely distributed plant in Vietnam and other Asian countries. It is about 4-6 m high with obliquely ovate acute and distichous thin leaves. The leaf is analgesic, antipyretic, antirheumatic and cures jaundice, small pox, itch and gum infection. Gooseberry plants start 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, there is a substantial fruit drop (50-75 %) at pre-harvest stage, its yield being 2-3 kg/plant during each flush. Fruits are pendulous, in small clusters from the branches, round or slightly flattened at the poles with shallow ribs of 0.75 inch across. 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 (Kundan kishore *et al.*, 2005). Gooseberry is one of the most important medicinal plants in traditional systems of medicine as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic, ulcer preventive and for common cold, fever; as alone or in combination with other plants (Y. Amirazodi et al., 2017). Phytochemical studies on gooseberry disclosed major chemical constituents including tannins, alkaloids, polyphenols, vitamins and minerals. Gallic acid, ellagic acid, emblicanin A & B, phyllembein, quercetin and ascorbic acid are found to be biologically effective (Swetha Dasaroju, Krishna Mohan Gottumukkala, 2014). The leaves of *P. acidus* have been used as anti-hypertensive remedy to relief headache resulting from hypertension (Chongsa *et al.*, 2014). In addition, *P. acidus* can improve eyesight problem, cure cough, and reduce severity of psoriasis, skin disorders and sudorifc (Chakraborty *et al.*, 2012). A study evaluated 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) (Tahira Foyzun et al., 2016).

Wine is one of the functional fermented foods that have many health benefits. Commercially, wine is produced by the fermentation of yeast which involves the conversion of sugar to alcohol. Wine can act as a nutrient supplement for seasonal fruits and vegetables throughout the year. Using fruits and vegetables having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely. Fermentation is carried out with Saccharomyces cerevisiae commonly known as bakers yeast. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. During the fermentation period the wines were analyzed for pH, titratable acidity, specific gravity, biomass content, alcohol and reducing sugar on a daily basis. 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 (Giri Nandagopal.M.S, Praveen.S.Nair, 2013). In the present, home-made wine production has been used various fruits including banana, apple, pineapple, cherry, berry, banana, cashew apple, pawpaw, water melon and orange (Obaedo and Ikenebomeh, 2009; Archibong *et al.*, 2015; Ogodo *et al.*, 2015; Lowor *et al.*, 2016) or local fruits which obtained the different flavor, aroma and taste based on type of fruit.

The alcohol content of home-made wines is only about 7-8% which makes it consumable for persons of any age group. Wine has great health benefits similar to those of fruits from which they are derived (Pongkan, S. et al., 2018). The yeast is responsible for the production of ethanol in alcoholic drink. The process produces ethyl alcohol (ethanol) is the way of yeast to convert glucose into energy. Fermentation can extract valuable components from the raw materials used for production. Yeast is the magical ingredient that turns fruit juices into wine. In spontaneous fermentations, the 1st stages invariably being dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae*. This species is universally known as the `wine yeast' and is widely preferred for initiating wine fermentations *S. cerevisiae* has adapted in several important ways and be able to break down their foods through both aerobic respiration and anaerobic fermentation. It can survive in an oxygen deficient environment for a period of time (Phonesavard Sibounnavong et al., 2010).The use of *S. cerevisiae* as starter culture is the most widespread practice in winemaking. However, the inoculation of musts using selected *Saccharomyces* strains does not ensure their dominance at the end of fermentation (Capece et al., 2010). In fact, although possessing high competition, commercial strains do not completely inhibit wild strains until several days after the process has started. The starter culture should compete with not only non-Saccharomyces yeasts, but also with indigenous S. cerevisiae strains, which theoretically adapt better to must conditions (Barrajón et al., 2011; Capece et al., 2011).

There were several studies mentioned to wine fermentation of gooseberry. A study was carried out to develop amla wine to minimize losses due to improper handling and unmarketability of fruits (A. Harshvardhan Reddy and V. Chikkasubbanna, 2010). Application of *Saccharomyces cerevisiae* for wine production from star gooseberry and carambola was mentioned. The experiment was to produce wine from star gooseberry by fermented with *Saccharomyces cerevisiae* for two weeks (Phonesavard Sibounnavong et al., 2010). Production of Wine from Ginger and Indian Gooseberry was conducted (Giri Nandagopal. M. S, Praveen. S. Nair, 2013). The amla wine was prepared by using *Saccharomyces cerevisiae* as a fermenter with addition of jaggery. The self generated alcohol content as ethanol was observed to be 9.29% (Argade V P, Pande V V, 2015). Influence of different fermentation conditions on the formulation and development of Amla (*Emblica officinalis* Gaertin.) wine was mentioned (Vaishali Punjahari Argade & Vishal Vijay Pande, 2016). In another research, wine was prepared from three varieties of *Phyllanthus* viz., *P. emblica* (wild and cultivated) and *P. acidus*. Fermentation induced changes in bioactive properties of wine from *Phyllanthus* with respect to atherosclerosis (Sinjitha S. Nambiar et al., 2016). Amla fruits can be used as a valuable ingredient for the production of an amla wine with all the important properties of wine having medicinal characteristics of amla fruits (Sanjay H. Amaley et al., 2016). A study on the preparation of wine from Amla (*Emblica ofcinalis* Gaertn.) using varied levels of sugar was carried out. The wine prepared using Amla fruits with 28°Brix sugar syrup were best out of all treatments (Adria Sarkar and Ashna Singhal, 2018).

Wine stimulates the release of digestive enzymes, which digest not only the alcohol but the many other nutrients found in wine. The proper dosage, or a moderate intake of wine, in addition to affecting cholesterol levels favourably, decreases the tendency of blood to clot and assists in dissolving clots, all important factors in protecting against heart disease. Research also indicates that moderate wine drinking may reduce the tendency of arteries to constrict during stress, lower blood pressure, and increase coronary artery diameter and blood flow. More recently, wine has been identified as a dependable source of quercetin, a potent anti-carcinogen, and of many flavonoids and other polyphenolic antioxidants (Giri Nandagopal.M.S, Praveen.S.Nair, 2013). Gooseberry is an underutilized fruit crop and still now there is very limited research available regarding to processing of this fruit into value added product. Therefore, we utilized this fruit as subtrate for wine fermentation. We focused on the effect of different parameters such as pectinase concentration and time of treatment for juice extraction, yeast inculate for wine fermentation, and secondary fermentation to wine quality.

2. MATERIAL & METHOD

2.1 Material

Gooseberry fruits were collected from Soc Trang province, Vietnam. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Gooseberry pulp was mixed with pectinase and distilled water ready for juice extraction. Total soluble solid (TSS) was adjusted to 20°Brix. Gooseberry juice was steriled by boiling for 5 minutes. *Saccharomyces cerevisiae* were maintained on potato dextrose agar (PDA) slant and kept at 4°C for further experiments. They were separately grown on PDA agar plates at 28°C for 48 hours. Next, they were separately transferred into 500-ml Erlenmeyer flask containing 250 ml of steriled gooseberry juice with 20°Brix TSS, pH 4.2. Then these flasks were incubated at 28°C under shaking condition at a speed of 40 rpm for 24 hours and used as inoculum. For fermentation of wine 10 ml of inoculum was transfer into 500-ml Erlenmayer flasks containing 390 ml of steriled fermentation medium. The flasks were incubated under static condition at room temperature (28°C) for 10 days.

Figure 1. Gooseberry (*Phyllanthus acidus)* fruit

2.2 Research method

2.2.1 Effect of pectinase concentration and time for juice extraction

Gooseberry extract was treated with pectinase enzyme with different concentration (1.0, 1.5, 2.0, 2.5%) in different duration (20, 30, 40, 50 minutes). We analyzed the extract recovery (%), viscosity (cP) and turbidity (mJ/cm^2).

2.2.2 Effect of yeast inculate for wine fermentation

Gooseberry wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.5, 1.0, 1.5, 2.0%). After 10 days of fermentation at 28^{o}C, we analyzed the soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine.

2.2.3 Effect of secondary fermentation to wine quality

We preserved Gooseberry wine at 9.5^{o}C in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored soluble dry matted (^{o}Brix), ethanol (% v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine.

2.3 Analysis of gooseberry wine

The viscosity of the samples was measured with Ostwalds's viscometer. Treated juices were kept overnight at room temperature (28°C) and were analysed for relative viscosity and turbidity, as a measure of clarification. Soluble dry matter (^{o}Brix) was measured by refractometer. Ethanol (% v/v) was determined by megapore polar column with direct injection gas chromatography (Mei-Ling Wang et al., 2003). Acidity (g/l) was measured by potentiometry method (M. B. Rajković et al., 2007). Total phenolic compounds (mg/g) in the extracts were determined using Folin–Ciocalteu reagent. The content of total phenolics was expressed as gallic acid equivalents (GAE). The spectrophotometer assay for the quantitative determination of flavonoid content (mg/g) was carried out. Total flavonoids (mg/g) of fruits were expressed as catechin equivalents. Sensory evaluation was carried out by a panel of 10 semi-trained judges.

2.4 Statistical analysis

Data were statistically summarized by Statgraphics Centurion XVI.

3. Result & Discussion

3.1 Effect of pectinase concentration and time of treatment for juice extraction

Pectinase enzyme which includes pectin methyl esterase and depolymerising enzymes, finds extensive application in fruit processing industries for clarification of fruit juices and wines, in the extraction of fruit juices, in the manufacturing of pectin free starch, curing of coffees, cocoa and tobacco, refinement of vegetable fibres, scouring and as an analytical tool for the estimation of plant products (Joshi VK and Bhutani VP, 1991; Tzanov T et al., 2001; Evans JD et al., 2002). The enzymatic liquefaction process not only helped in increasing the overall yield of juice but also upgrading the quality features of the extracted juice leading to sparkling clarity (Sakhale, B. K. et al. 2016). Gooseberry extract was treated with pectinase enzyme with different concentration (1.0, 1.5, 2.0, 2.5%) in different duration (20, 30, 40, 50 minutes). Our results were depicted in table 1, 2 and 3. We clearly found that 2.0% pectinase in 40 minutes treatment was optimal for Gooseberry extraction. So we selected these values for next experiments.

Table 1. Extract recovery (%) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Extract recovery (%)			
	20 minutes	30 minutes	40 minutes	50 minutes
1.0	51.45±0.03^b	52.36±0.02^b	53.29±0.03^b	53.32±0.01^b
1.5	53.28±0.04^{ab}	54.13±0.03^{ab}	55.04±0.01^{ab}	55.11±0.02^{ab}
2.0	55.19±0.02^a	56.49±0.04^a	57.58±0.04^a	57.63±0.00^a
2.5	55.22±0.01^a	56.62±0.01^a	57.65±0.02^a	57.80±0.03^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%).

Table 2. Viscosity (cP) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Viscosity (cP)			
	20 minutes	30 minutes	40 minutes	50 minutes
1.0	1.54±0.04^a	1.41±0.04^a	1.37±0.00^a	1.35±0.02^a
1.5	1.12±0.00^b	1.10±0.02^b	1.03±0.03^{bc}	1.00±0.04^b
2.0	1.04±0.01^{bc}	1.01±0.01^{bc}	0.90±0.02^b	0.82±0.01^{bc}
2.5	0.95±0.02^c	0.82±0.03^c	0.79±0.04^c	0.75±0.00^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%).

Table 3. Turbidity (mJ/cm^2) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Optical density (mJ/cm^2)			
	20 minutes	30 minutes	40 minutes	50 minutes
1.0	73.32±0.04^a	71.36±0.00^a	70.24±0.03^a	70.15±0.00^a
1.5	70.56±0.02^b	70.41±0.04^b	70.22±0.00^b	69.99±0.02^b
2.0	69.13±0.01^{bc}	69.02±0.02^{bc}	68.77±0.02^{bc}	68.54±0.01^{bc}
2.5	69.08±0.00^c	68.94±0.01^c	68.69±0.04^c	68.48±0.03^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%).

The enzymatic liquefaction of pulp as a function of enzyme concentration, incubation time and hydrolysing temperature is standardized to obtain a desired yield of brilliantly cleared juice (Bhattacharya and Rastogi, 1999). The effects of pectinase concentration and biocatalytic time on the content of ascorbic acid, phenolic compounds and antioxidant activity of the fruit juice were firstly investigated. Response surface methodology was then used to optimize the conditions of enzymatic extraction for maximizing the antioxidant activity of the star gooseberry juice (Do Thi Thanh Loan et al., 2017). For extraction of juice, 2.5% enzyme concentration was found to be the best. However, for clarification of apple and pear juice 1.0 and 0.5% concentration, respectively gave optimum results (V K Joshi et al., 2011).

3.2 Effect of yeast inculate for wine fermentation

In pure fermentation, the ability of inoculated *Saccharomyces cerevisiae* to suppress the wild microflora is one of the most important feature determining the starter ability to dominate the process. During the winemaking process, various microorganisms coexist and interact influencing the dominance, the persistence of fermenting yeasts and the analytical profiles of wine (Maurizio Ciani et al., 2016).

Gooseberry wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.5, 1.0, 1.5, 2.0%). After 10 days of fermentation at 28^{o}C, we noticed the change of soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine as in table 4, 5, 6, 7, 8, 9. We found that the appropriate yeast inculate should be 1.5% to get the highest wine quality.

Effect of different inoculum concentrations indicated that increased the inoculum concentration result in the increased of alcohol content. The result showed that when the concentration of yeast was increased, yeast cells converted more sugar to alcohol. However, at the higher inoculum concentration yeast cells grew not well because of the limited nutrient and were not able to convert more sugar in to it (Pongkan, S. et al., 2018). The results obtained were agreed with the report of Satav and Pethe (2017) who studied wine production from banana fruits. In this study, 10% and 15% inoculum concentration gave similar alcohol content but 10% showed the better taste than 15%. The 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. It is demonstrated that all formulations of star gooseberry wine showed significantly higher amount of ethyl alcohol than all formulations of carambola wine (Phonesavard Sibounnavong et al., 2010).

3.3 Effect of secondary fermentation to wine quality

During maturation, aging and storage of wine, coloured and noncoloured phenolics have an important role on the colour and taste of wine and they undergo a number of reactions during aging that result in changes of the sensory characteristics. We preserved Gooseberry wine at 9.5^{o}C in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored soluble dry matted (^{o}Brix), ethanol (% v/v), acidity (g/l), and sensory characteristics (score) in wine. Our results were elaborated in table 10. We noted that the longer of the secondary fermentation, the better of wine quality we got. However, there was not significant change of samples being preserved at the 3rd and 4th week so we choosed 3 weeks of secondary fermentation for economy.

Table 4. Effect of yeast ratio to soluble dry matter (^{o}Brix) in wine

Fermentation time (days)	Soluble dry matter in wine (^{o}Brix)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	17.45±0.04^{a}	14.21±0.02^{b}	12.13±0.03^{bc}	12.01±0.03^{c}
2	15.29±0.00^{a}	12.68±0.04^{b}	10.79±0.01^{bc}	10.65±0.02^{c}
3	13.17±0.01^{a}	10.43±0.01^{b}	9.78±0.03^{bc}	9.54±0.04^{c}
4	12.28±0.03^{a}	9.85±0.03^{b}	8.50±0.02^{bc}	8.42±0.04^{c}
5	11.04±0.02^{a}	8.21±0.02^{b}	7.42±0.04^{bc}	7.31±0.02^{c}
6	10.18±0.00^{a}	7.39±0.04^{b}	6.19±0.02^{bc}	6.04±0.03^{c}
7	9.23±0.04^{a}	6.43±0.02^{b}	5.48±0.0^{bc}	5.37±0.02^{c}
8	8.16±0.02^{a}	5.17±0.01^{b}	4.17±0.02^{bc}	4.05±0.04^{c}
9	7.21±0.00^{a}	4.22±0.02^{b}	3.95±0.04^{bc}	3.78±0.02^{c}
10	5.98±0.03^{a}	3.19±0.01^{b}	2.41±0.01^{bc}	2.35±0.03^{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%).

Table 5. Effect of yeast ratio to ethanol formation (%v/v) in wine

Fermentation time (days)	Ethanol in wine (%v/v)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	2.36±0.03^{b}	3.65±0.02^{ab}	4.87±0.04^{a}	4.95±0.04^{a}
2	2.78±0.01^{b}	3.96±0.02^{ab}	5.26±0.02^{a}	5.32±0.01^{a}
3	3.01±0.02^{b}	4.45±0.04^{ab}	5.87±0.01^{a}	5.94±0.02^{a}
4	3.48±0.04^{b}	4.97±0.03^{ab}	6.32±0.02^{a}	6.39±0.04^{a}
5	3.95±0.04^{b}	5.24±0.00^{ab}	6.91±0.02^{a}	7.02±0.02^{a}
6	4.28±0.02^{b}	5.87±0.00^{ab}	7.27±0.03^{a}	7.31±0.03^{a}
7	4.86±0.03^{b}	6.12±0.03^{ab}	7.75±0.02^{a}	7.84±0.04^{a}
8	5.20±0.02^{b}	6.73±0.02^{ab}	7.98±0.02^{a}	8.03±0.01^{a}
9	5.76±0.02^{b}	6.98±0.03^{ab}	8.24±0.03^{a}	8.33±0.01^{a}
10	6.00±0.04^{b}	7.15±0.02^{ab}	8.53±0.04^{a}	8.59±0.03^{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%).

Table 6. Effect of yeast ratio to acidity (g/l) in wine

Fermentation time (days)	Acidity in wine (g/l)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	1.02±0.03^{b}	1.06±0.04^{ab}	1.17±0.01^{a}	1.20±0.02^{a}
2	1.09±0.01^{b}	1.10±0.00^{ab}	1.21±0.03^{a}	1.23±0.02^{a}
3	1.14±0.00^{b}	1.16±0.02^{ab}	1.30±0.01^{a}	1.32±0.01^{a}
4	1.19±0.04^{b}	1.22±0.03^{ab}	1.39±0.00^{a}	1.40±0.04^{a}
5	1.23±0.02^{b}	1.27±0.01^{ab}	1.47±0.00^{a}	1.49±0.02^{a}
6	1.37±0.01^{b}	1.40±0.04^{ab}	1.62±0.04^{a}	1.65±0.01^{a}
7	1.43±0.03^{b}	1.48±0.01^{ab}	1.74±0.01^{a}	1.76±0.03^{a}
8	1.51±0.02^{b}	1.55±0.02^{ab}	1.80±0.02^{a}	1.82±0.01^{a}
9	1.62±0.01^{b}	1.69±0.03^{ab}	1.91±0.03^{a}	1.93±0.02^{a}
10	1.73±0.04^{b}	1.75±0.01^{ab}	1.98±0.04^{a}	2.00±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%).

Table 7. Effect of yeast ratio to total phenolic content (mg GAE/g) in wine

Fermentation time (days)	Total phenolic content (mg GAE/g)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	184.79±0.02^{b}	198.45±0.02^{ab}	214.05±0.01^{a}	216.11±0.04^{a}
2	191.23±0.03^{b}	203.27±0.02^{ab}	222.38±0.04^{a}	223.04±0.03^{a}
3	198.27±0.00^{b}	209.34±0.04^{ab}	229.45±0.03^{a}	230.05±0.01^{a}
4	202.13±0.04^{b}	211.28±0.02^{ab}	237.18±0.01^{a}	237.43±0.03^{a}
5	207.49±0.02^{b}	218.55±0.01^{ab}	241.53±0.00^{a}	242.00±0.04^{a}
6	211.38±0.01^{b}	232.01±0.00^{ab}	249.40±0.04^{a}	249.83±0.01^{a}
7	219.42±0.04^{b}	247.53±0.04^{ab}	265.15±0.01^{a}	265.79±0.03^{a}
8	233.05±0.02^{b}	252.18±0.01^{ab}	274.39±0.02^{a}	275.00±0.04^{a}
9	241.16±0.04^{b}	260.41±0.03^{ab}	285.10±0.04^{a}	285.73±0.02^{a}
10	247.95±0.02^{b}	271.04±0.01^{ab}	294.27±0.03^{a}	294.68±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%).

Table 8. Effect of yeast ratio to total flavonoid (mg CE/g) in wine

Fermentation time (days)	Total flavonoid (mg CE/g)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	11.25±0.01^{b}	12.38±0.01^{ab}	13.69±0.01^{a}	13.74±0.00^{a}
2	11.49±0.04^{b}	12.94±0.03^{ab}	14.10±0.03^{a}	14.22±0.02^{a}
3	11.63±0.03^{b}	13.38±0.02^{ab}	14.75±0.01^{a}	14.81±0.02^{a}
4	11.84±0.04^{b}	13.85±0.03^{ab}	15.03±0.02^{a}	15.09±0.01^{a}
5	11.99±0.02^{b}	14.03±0.00^{ab}	15.29±0.04^{a}	15.33±0.03^{a}
6	12.14±0.03^{b}	14.38±0.03^{ab}	15.87±0.00^{a}	15.92±0.01^{a}
7	12.78±0.04^{b}	14.64±0.01^{ab}	16.22±0.03^{a}	16.25±0.03^{a}
8	12.94±0.01^{b}	14.93±0.02^{ab}	16.78±0.01^{a}	16.83±0.00^{a}
9	13.03±0.02^{b}	15.21±0.04^{ab}	17.33±0.02^{a}	17.36±0.04^{a}
10	13.16±0.03^{b}	15.68±0.00^{ab}	18.04±0.01^{a}	18.09±0.02^{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%).

Table 9. Effect of yeast ratio to sensory characteristics (score, 1-5) in wine

Fermentation time (days)	Sensory score of wine (1-5) by different yeast ratio			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	2.24±0.03^{b}	2.36±0.03^{ab}	2.42±0.03^{a}	2.48±0.04^{a}
2	2.31±0.01^{b}	2.54±0.02^{ab}	2.64±0.01^{a}	2.67±0.02^{a}
3	2.55±0.04^{b}	2.87±0.02^{ab}	2.95±0.03^{a}	3.02±0.00^{a}
4	2.63±0.00^{b}	2.98±0.04^{ab}	3.11±0.01^{a}	3.15±0.01^{a}
5	2.92±0.00^{b}	3.15±0.02^{ab}	3.64±0.02^{a}	3.69±0.02^{a}
6	3.11±0.01^{b}	3.42±0.01^{ab}	3.85±0.03^{a}	3.90±0.00^{a}
7	3.37±0.03^{b}	3.83±0.01^{ab}	4.01±0.04^{a}	4.03±0.01^{a}
8	3.85±0.04^{b}	4.06±0.03^{ab}	4.14±0.00^{a}	4.17±0.03^{a}
9	4.03±0.02^{b}	4.39±0.04^{ab}	4.42±0.01^{a}	4.45±0.02^{a}
10	4.14±0.01^{b}	4.52±0.01^{ab}	4.64±0.04^{a}	4.67±0.00^{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%).

Table 10. Effect of the sencondary fermentation to wine quality

Criteria	Secondary fermentation (weeks)			
	1	2	3	4
Soluble dry matter (^{o}Brix)	2.39±0.01^{a}	2.31±0.01^{ab}	2.27±0.02^{ab}	2.23±0.03^{b}
Ethanol (%v/v)	8.60±0.01^{b}	8.71±0.03^{ab}	8.82±0.03^{ab}	8.89±0.04^{a}
Acidity (g/l)	1.98±0.00^{a}	1.96±0.04^{ab}	1.95±0.01^{ab}	1.93±0.02^{b}
Total phenolic content (mg GAE/g)	295.44±0.03^{a}	292.31±0.02^{ab}	291.02±0.01^{ab}	290.17±0.02^{b}
Total flavonoid (mg CE/g)	18.11±0.01^{a}	18.06±0.02^{ab}	18.03±0.01^{ab}	18.00±0.01^{b}
Sensory score	4.65±0.02^{b}	4.71±0.02^{ab}	4.79±0.04^{ab}	4.85±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%).

All the useful natural components of amla, Indian gooseberry, *Emblica officinalis* Gaertn., with therapeutic value, can be easily extracted in water after dispensing the berries in hot water. Ameliorating the extract, with the sugar made it a good medium for the growth of *Saccharomyces cerevisiae* and fermenting the sugar into ethanol to make wine. The wine was found similar to any other wine in terms of its composition, taste and aroma. The conditions for achieving the highest alcohol content and improving the sensory qualities have been standardized by evaluating the effect of addition of various exogenous nutrients, environmental conditions, fermentation technology and by maturing the wine. The supplementation of ammonium sulphate, potassium dihydrogen phosphate, proline and biotin to the hot water extract of amla proved to be best nutritional factors for highest alcohol production (12%) during the fermentation of the amla based medium with a new strain of *S. cerevisiae* in a batch fermentation. The alcohol content was further improved to 16.1% in a fed batch fermentation involving the repeated feeding of sugar for 2 cycles after an interval of 3 days each in a batch where the initial TSS was maintained at 20% and the feeding was done when the original TSS fell to 10% at each of two stages. Further, the storage of wine in oak wood barrel for a month improved its quality and led to the reduction in undesirable components such as n-propanol, n-butanol, iso-butanol, isoamyl alcohols and an increase in desirable components including ethyl acetate, phenolics (S K Soni et al., 2009). According to Violeta Ivanova et al., (2012), when polymerization of phenolics occurs, wine aging affected the phenolic content of wines produced with 3 days of maceration and caused intensive decrease of anthocyanins during the storage period. A research finding was to produce and improve the quality of tamarind wine. The optimal conditions for production of Gooseberry wine was 10% inoculum concentration, 5% tamarind juice and 20 °Brix total soluble solid with the 0.67 percentage of alcohol by volume and 3.63 ± 0.10 points from sensory evaluation **(**Pongkan, S. et al., 2018**).**

4. CONCLUSION

Wine is an alcoholic beverage producing by fermentation of yeast, *Saccharomyces cerevisiae* in fruit juice. In general, grape is the most popular fruit for wine production because grape juice is rich of carbon sources, nutrients and enzyme for yeast fermention. Yeast grows and converts sugar in fruit juices into alcohol and carbondioxide. We have successfully utilized Gooseberry as substrate for wine fermentation by investigating different parameters such as pectinase concentration and time of treatment for juice extraction, yeast inculate for wine fermentation, and secondary fermentation to wine quality. Wine has great health benefits similar to those of fruits from which they are derived.

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