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Different factors affecting Guava (*Psidium guajava*) wine fermentation

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Abstract.

Guava (*Psidium guajava*) is an evergreen shrub or small tree in the family Myrtaceae grown for its edible fruits. The fruit is prized for its very pleasant, sub acidic and aromatic nature. Guava possesses favourable nutritional characteristics as a source of phenolic compounds, carotenoids and vitamin C, excellent flavour, aroma and colour. Guava fruits are fresh during the harvesting season but perishable under the prevailing conditions of temperature and humidity as well as lack of adequate storage facilities. An alternative way of preserving surplus guava could be to ferment the juice to fruit wine. Therefore we explored a wine fermentation from guava 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 0.20% pectinase was used for juice extraction in 30 minutes, initial soluble dry matter 16^{o}Brix, 0.15% *sacchromyces cerevisiae* was used for the main fermentation at 11.5^{o}C, and 3 weeks of aging in dark bottle at 8.5^{o}C was applied to get a pleasant guava quality. By preparing guava wine as value added beverage, enhanced returns can be obtained by the growers.

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

1. INTRODUCTION

Guava *(Psidium guajava)* is an evergreen shrub or small tree in the family Myrtaceae grown for its edible fruits (Jyoti D. Vora et al., 2018). Guava (*Psidium guajava* Linn.) commonly known for its food and nutritional values throughout the world. The common types of guava include apple guava, yellow fruited cherry guava, strawberry guava, and red apple guava. The carbohydrate content in the white pulp guava was found to behigher than the red pulp guava. The protein content varied significantly with more amount of proteins in the red pulp guava. The amount of crude fibres in red pulp guava was more as compared to white pulp guava. Moisture content indicated that the red variety contains less amount of water. The mineral elements analysis indicated that the red pulp guava fruit was significantly higher in calcium, sodium and phosphorous. The white variety was found to be rich in potassium. Sodium content in white guava was in very minute quantity and hence was not detectable. The value of ascorbic acid was higher in red guava, which indicated that the red pulp variety is richer in vitamin C. Isolation of pectin displayed that the white variety of guava exhibited more amount of pectin than the red one (Jyoti D. Vora et al., 2018). Guayaba passes through different stages of harvesting and post-harvest conservation. It is mostly eaten raw (ripe or semi-ripe) or consumed in the form of juice, syrup, ice cream, jams, and jellies (M. Alejandra Moreno et al., 2014). The common guava has a fruit with a yellow skin and white, yellow, or pink flesh. Guavas are known for their sweet and tangy flavor and many uses. A number of chemicals isolated from plants like quercetin, guaijaverin, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, kaempferol flavonoids and galactose-specific lecithins have shown promising activity. Carotenoids and ascorbic acid were the dominant phytochemicals in flour as well as in fresh fruits. Toxicity studies in mice and other animal models as well as controlled human studies show leaf, seed, pulp, skin and fruits different extract in different concentration are helps to prevent cancer, regulating blood pressure, and treating diarrhea (Bruna Galdorfni Chiari-Andréo et al., 2017). Much of the traditional uses have been validated by scientific research. The plant has been extensively studied in terms of pharmacological activity of its major components and the results show antioxidant, antipyretic, antifungal, antimicrobial, hypotensive, analgesic & anti-inflammatory effect (Shakib Uzzaman et al., 2018). The presence of terpenes, caryophyllene oxide and *p*-selinene produces relaxation effects. Guava leaves contain many compounds which act as fungistatic and bacteriostatic agents. Guava has a high content of important antioxidants and has radio-protective ability. Quercetin is considered as most active antioxidant in the guava leaves and is responsible for its spasmolytic activity. Its ethyl acetate extract can stop the germ infection and thymus production. Guava possesses anti-viral, anti-inflammatory, anti-plaque and anti-mutagenic activities. Guava extract shows antinociceptive activity and is also effective in liver damage inflammation and serum production. Ethanolic extract of guava can increase the sperm quality as well as quantity and can be used for the treatment of infertile males (Sumra Naseer et al., 2018).

Guava has been nomenclature by many nutritionists as a “super fruit” due to its easy cultivation, availability and a countless list of health benefits. The medicinal properties of guava fruit, leaf and other parts of

the plant are also well known in traditional system of medicine. Since, each part of guava tree possesses economic value; it is grown on commercial scale. A very well-known nutritional benefit of consumption of guava is its rich Vitamin C content performing varied immune functions and protecting the body from free radicals. Apart from this, a high level of manganese, folate, and fibre have additional benefits that are associated with guava (Jyoti D. Vora et al., 2018). However, guayaba is a fruit highly perishable and susceptible to damage during the postharvest (M. Alejandra Moreno et al., 2014). Guava is an underutilized fruit crop and still now there is very limited research available regarding to processing of this fruit into value added product. Two different strains of *Saccharomyces cerevisiae* NCIM 3095 and NCIM 3287 were evaluated in the production of guava fruit wine (Sevda SB and Rodrigues L, 2011). Production of wine from over ripe guava (*Psidium guajava*) and ber (*Ziziphus mauritiana*) fruits using *Saccharomyces crevices* was conducted (Kaiser Younis et al., 2014). The potential of wine production from guava is presented (Singh E. and Puyo A., 2014). A study was conducted to produce wine from juice of guava (*Psidium guajava* L.) cv. Punjab Pink following treatment with pectinase enzyme (Pooja Nikhanj et al., 2017). The development and physicochemical and volatile characterization of a natural sparkling guava wine produced by the champenoise method was conducted (Silvana Maria Michelin Bertagnolli et al., 2017).

The guava fruit, which typically has high fermentable sugar composition when mature and ripe, could be exploited as a substrate for alcoholic fermentation. High rate wastage of this fruit especially at its peak of production season necessitates the need for alternative preservation and post harvest technologies towards its value addition that can reduce the level of post harvest losses besides increasing diversity of wine. 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

We collected guava in Ke Sach district, Soc Trang province, Vietnam. They must be cultivated following VietGAP without pesticide and fertilizer residue to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Apart from collecting guava, we also used other materials such as pectinase, yeast. Lab utensils and equipments included knife, weight balance, fermentation tank, refractometer, viscometer, flow UV system, pH meter, ethanol meter, buret.

Ripened guava fruits were selected after manual sorting, washed in hot water, cut into pieces, dipped in water and pasteurized for 30-40 min. Thereafter, softened fruit pieces were squeezed and filtered through muslin cloth to obtain juice that was stored in flasks under refrigerator conditions (8°C) till further use. Pre-fermentation treatment of guava must with pectinase enzyme. Inoculum of *Saccharomyces cerevisiae* for carrying out ethanolic fermentation of pretreated guava juice was prepared in glucose yeast extract broth where a loopful of slant culture was inoculated in 250 ml Erlenmeyer flasks containing 100 ml glucose yeast extract. Pretreated guava juice was taken in fermenters to produce the wine. The fermented wort was subjected to storage for settling of yeast and other biological or chemical debris.

Figure 1. Guava *(Psidium guajava)*

2.2 Research method

2.2.1 Effect of pectinase concentration and time for juice extraction

Guava extract was treated with pectinase enzyme with different concentration (0.10, 0.15, 0.20, 0.25%) 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 initial soluble dry matter for wort fermentation

The extracted juice was formulated in different concentration of the initial soluble dry matter (14, 15, 16, 17 ^oBrix). After 9 days of fermentation at 11.5^oC, we analyzed the residual soluble dry matter (^oBrix), ethanol (%v/v), acidity (g/l), and sensory characteristics (score) in wort.

2.2.3 Effect of yeast inculate for wort fermentation

Guava wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.05, 0.1, 0.15, 0.2%). After 9 days of fermentation at 11.5^oC, we analyzed the residual soluble dry matter (^oBrix), ethanol (%v/v), acidity (g/l), and sensory characteristics (score) in wine.

2.2.3 Effect of secondary fermentation to wine quality

We preserved guava wine at 8.5^oC in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored residual soluble dry matted (^oBrix), ethanol (% v/v), acidity (g/l), and sensory characteristics (score) in wine.

2.3 Physico-chemical and sensory evaluation

Soluble dry matter (^oBrix) was measured by refractometer. Ethanol (% v/v) was analyzed by by GLC (Antony 1984). Acidity (g/l) was measured by potentiometric titration using an ion-selective electrode (M. B. Rajković et al., 2007). Sensory score was based on 5-point hedonic scale.

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

Alcoholic fermentation is a combination of complex interactions involving must variety, micro biota and

winemaking technology. Some factors strongly affect alcoholic fermentation, and consequently the quality of the wine. The most important factors are the clarification of the juice, the temperature of fermentation, the composition of the juice, inoculation with selected yeasts and the interaction with other microorganisms (Musyimi S.M et al., 2013). Juice clarification is an important pre-fermentation step for effective extraction and clarification of guava juice since it contains high amount of pectins. Specific enzymes and enzyme combinations are required to optimize the extraction of any particular fruit juice concerning its yield and quality. Among these enzymes, pectinases can hydrolyze pectin and cause pectin-protein complexes to flocculate, so that the resulting juice has much lower amount of pectin and low viscosity, which is advantageous for the filtration process (Rai et al., 2004). Guava extract was treated with pectinase enzyme with different concentration (0.10, 0.15, 0.20, 0.25%) in different duration (20, 30, 40, 50 minutes). Our results were depicted in table 1, 2 and 3. We clearly found that 0.2% pectinase in 30 minutes treatment was optimal for guava extraction. So we selected these values for next experiments.

The potential of wine production from guava is presented. The chaptalized juice ("must") is treated with pectinase or a combination of enzymes and fermented with traditional yeasts at a temperature range of 22 to 30°C and inoculum size of 6 to 11% (v/v). The addition of N and P improves ethanol production and quality parameters of guava wine. Racking and ageing of guava wine also improves the sensory and organoleptic characteristics of guava wine (Singh E. and Puyo A., 2014). A study was conducted to produce wine from juice of guava (*Psidium guajava* L.) cv. Punjab Pink following treatment with pectinase enzyme. It was observed that pre-fermentative treatment of 'must' with 0.50 mg/100ml pectinase at 45°C for 6h resulted in 47.2% clarity of guava juice. (Pooja Nikhanj et al., 2017)

3.2 Effect of initial soluble dry matter for wort fermentation

The extracted juice was formulated in different concentration of the initial soluble dry matter (14, 15, 16, 17 ^{o}Brix). After 9 days of fermentation at 11.5^{o}C, we analyzed the residual soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), and sensory characteristics (score) in wort in table 4, 5, 6 and 7. We found that the appropriate yeast inculate should be 0.15% to get the highest wort quality.

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
0.10	64.39±0.03^{b}	64.95±0.02^{b}	65.37±0.03^{b}	65.71±0.01^{b}
0.15	66.53±0.01^{ab}	66.79±0.02^{ab}	67.25±0.01^{ab}	67.38±0.03^{ab}
0.20	67.48±0.03^{a}	67.92±0.01^{ab}	68.36±0.00^{ab}	67.70±0.01^{ab}
0.25	67.60±0.02^{a}	68.10±0.03^{a}	68.83±0.01^{a}	68.94±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 2. Viscosity (cP) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Viscosity (cP)			
	20 minutes	30 minutes	40 minutes	50 minutes
0.10	1.07±0.02^{a}	0.95±0.02^{a}	0.83±0.01^{a}	0.78±0.02^{a}
0.15	0.94±0.01^{ab}	0.88±0.00^{ab}	0.81±0.02^{a}	0.75±0.01^{a}
0.20	0.87±0.00^{ab}	0.74±0.01^{ab}	0.69±0.02^{ab}	0.67±0.02^{ab}
0.25	0.79±0.02^{b}	0.72±0.01^{b}	0.65±0.01^{b}	0.64±0.00^{b}

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
0.10	68.63±0.03^{a}	66.72±0.02^{ab}	65.41±0.01^{ab}	64.04±0.01^{b}
0.15	66.32±0.00^{a}	65.79±0.00^{bb}	64.15±0.00^{ab}	63.26±0.02^{b}
0.20	65.11±0.03^{a}	64.36±0.01^{ab}	63.17±0.01^{ab}	62.58±0.00^{b}
0.25	63.49±0.01^{a}	62.77±0.01^{ab}	62.25±0.00^{ab}	62.11±0.02^{b}

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 4. Effect of initial soluble dry matter (14, 15, 16, 17^{o}Brix) to residual soluble dry matter (^{o}Brix) in wort

Fermentation time (days)	**Residual soluble dry matter in wort (^{o}Brix)**			
	14^{o}Brix	15^{o}Brix	16^{o}Brix	17^{o}Brix
1	13.55±0.03^{a}	14.58±0.01^{b}	15.22±0.01^{c}	16.01±0.02^{d}
2	13.06±0.01^{ab}	14.03±0.03^{b}	14.88±0.02^{c}	15.66±0.01^{d}
3	12.89±0.00^{b}	13.74±0.02^{b}	14.13±0.00^{c}	14.89±0.00^{d}
4	12.35±0.02^{bc}	13.11±0.02^{b}	13.89±0.03^{c}	14.12±0.01^{d}
5	11.46±0.01^{c}	12.85±0.01^{b}	13.23±0.01^{c}	13.85±0.00^{d}
6	11.04±0.03^{cd}	12.30±0.02^{b}	12.70±0.00^{c}	13.28±0.02^{d}

7	10.39±0.02^{d}	12.00±0.01^{b}	12.02±0.01^{c}	12.86±0.00^{d}
8	10.02±0.00^{de}	11.74±0.02^{b}	11.86±0.03^{c}	12.30±0.01^{d}
9	9.57±0.02^{e}	11.06±0.01^{b}	11.17±0.01^{c}	12.02±0.02^{d}
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 initial soluble dry matter (14, 15, 16, 17^{o}Brix) to ethanol formation (%v/v) in wort

Fermentation time (days)	**Ethanol in wort (%v/v)**			
	14^{o}Brix	15^{o}Brix	16^{o}Brix	17^{o}Brix
1	2.38±0.02^{c}	3.02±0.03^{b}	3.78±0.00^{ab}	3.94±0.02^{a}
2	2.89±0.00^{c}	3.48±0.00^{b}	3.99±0.03^{ab}	4.02±0.01^{a}
3	3.40±0.01^{c}	3.97±0.00^{b}	4.35±0.01^{a}	4.14±0.00^{ab}
4	3.94±0.02^{c}	4.24±0.02^{b}	4.88±0.02^{a}	4.34±0.03^{ab}
5	4.55±0.01^{c}	4.75±0.02^{b}	5.19±0.03^{a}	4.87±0.02^{ab}
6	4.96±0.02^{c}	5.16±0.01^{ab}	5.85±0.01^{a}	5.01±0.01^{b}
7	5.23±0.00^{b}	5.84±0.02^{ab}	6.18±0.02^{a}	5.17±0.03^{c}
8	5.69±0.01^{bc}	6.03±0.01^{b}	6.77±0.01^{a}	5.23±0.02^{c}
9	5.90±0.02^{c}	6.37±0.00^{b}	6.94±0.03^{a}	5.44±0.01^{d}
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 initial soluble dry matter (14, 15, 16, 17^{o}Brix) to acidity (g/l) in wort

Fermentation time (days)	**Acidity in wort (g/l)**			
	14^{o}Brix	15^{o}Brix	16^{o}Brix	17^{o}Brix
1	1.02±0.01^{c}	1.06±0.02^{b}	1.11±0.01^{a}	1.08±0.03^{ab}
2	1.03±0.02^{c}	1.08±0.01^{b}	1.15±0.02^{a}	1.10±0.01^{ab}
3	1.06±0.03^{c}	1.11±0.03^{b}	1.18±0.01^{a}	1.13±0.03^{ab}
4	1.08±0.02^{c}	1.12±0.00^{b}	1.20±0.03^{a}	1.17±0.01^{ab}
5	1.12±0.02^{c}	1.15±0.02^{b}	1.23±0.01^{a}	1.19±0.01^{ab}
6	1.14±0.01^{c}	1.18±0.00^{b}	1.28±0.02^{a}	1.23±0.02^{ab}
7	1.17±0.03^{c}	1.23±0.02^{b}	1.31±0.00^{a}	1.28±0.01^{ab}
8	1.21±0.00^{c}	1.26±0.03^{b}	1.35±0.02^{a}	1.30±0.00^{ab}
9	1.24±0.01^{c}	1.30±0.01^{b}	1.37±0.03^{a}	1.32±0.01^{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%).				

Table 7. Effect of initial soluble dry matter (14, 15, 16, 17^{o}Brix) to sensory characteristics in wort

Fermentation time (days)	**Sensory score of wort (1-5) by different initial soluble dry matter**			
	14^{o}Brix	15^{o}Brix	16^{o}Brix	17^{o}Brix
1	2.21±0.01^{c}	2.54±0.03^{b}	2.78±0.01^{a}	2.82±0.02^{ab}
2	2.29±0.02^{c}	2.64±0.01^{b}	2.84±0.00^{a}	2.93±0.01^{ab}
3	2.48±0.03^{c}	2.86±0.01^{b}	2.98±0.03^{a}	2.97±0.00^{ab}
4	2.73±0.01^{c}	2.94±0.02^{b}	3.12±0.00^{a}	3.03±0.02^{ab}
5	2.88±0.02^{c}	2.99±0.01^{b}	3.17±0.01^{a}	3.05±0.01^{ab}
6	3.09±0.01^{c}	3.14±0.00^{b}	3.78±0.02^{a}	3.60±0.01^{ab}
7	3.19±0.03^{c}	3.48±0.02^{b}	3.92±0.03^{a}	3.80±0.02^{ab}
8	3.77±0.00^{c}	3.95±0.01^{b}	4.13±0.01^{a}	4.02±0.03^{ab}
9	3.92±0.02^{c}	4.01±0.03^{b}	4.19±0.03^{a}	4.05±0.01^{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%).				

Table 8. Effect of yeast ratio to residual soluble dry matter (^{o}Brix) in wort

Fermentation time (days)	**Soluble dry matter in wort (^{o}Brix)**			
	Yeast ratio 0.05%	Yeast ratio 0.10%	Yeast ratio 0.15%	Yeast ratio 0.20%
1	15.22±0.01^{a}	15.01±0.01^{ab}	14.84±0.02^{ab}	14.56±0.01^{b}
2	14.88±0.02^{a}	14.77±0.03^{ab}	14.62±0.00^{ab}	14.48±0.00^{b}
3	14.13±0.00^{a}	14.01±0.01^{ab}	13.88±0.00^{ab}	13.65±0.03^{b}
4	13.89±0.03^{a}	13.74±0.02^{ab}	13.55±0.03^{ab}	13.21±0.01^{b}
5	13.23±0.01^{a}	13.01±0.01^{ab}	12.89±0.01^{ab}	12.63±0.02^{b}
6	12.70±0.00^{a}	12.40±0.02^{ab}	12.04±0.01^{ab}	11.70±0.03^{b}
7	12.02±0.01^{a}	11.63±0.01^{ab}	11.04±0.02^{ab}	10.46±0.01^{b}
8	11.86±0.03^{a}	11.03±0.01^{ab}	10.78±0.01^{ab}	10.11±0.02^{b}
9	11.17±0.01^{a}	10.84±0.03^{ab}	10.03±0.02^{ab}	9.94±0.02^{b}
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 ethanol formation (%v/v) in wort

Fermentation time (days)	Ethanol in wort (%v/v)			
	Yeast ratio 0.05%	Yeast ratio 0.10%	Yeast ratio 0.15%	Yeast ratio 0.20%
1	3.78±0.00^{a}	3.94±0.03^{ab}	4.02±0.00^{ab}	4.11±0.01^{b}
2	3.99±0.03^{a}	4.23±0.00^{ab}	4.59±0.03^{ab}	4.75±0.02^{b}
3	4.35±0.01^{a}	4.88±0.00^{ab}	5.02±0.01^{ab}	5.09±0.00^{b}
4	4.88±0.02^{a}	5.13±0.02^{ab}	5.29±0.00^{ab}	5.38±0.01^{b}
5	5.19±0.03^{a}	5.47±0.02^{ab}	5.84±0.02^{ab}	5.92±0.02^{b}
6	5.85±0.01^{a}	6.14±0.00^{ab}	6.95±0.02^{ab}	7.00±0.03^{b}
7	6.18±0.02^{a}	6.87±0.01^{ab}	7.03±0.03^{ab}	7.16±0.01^{b}
8	6.77±0.01^{a}	6.98±0.02^{ab}	7.19±0.01^{ab}	7.23±0.03^{b}
9	6.94±0.03^{a}	7.20±0.01^{ab}	7.45±0.03^{ab}	7.50±0.01^{b}

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 yeast ratio to acidity (g/l) in wort

Fermentation time (days)	Acidity in wort (g/l)			
	Yeast ratio 0.05%	Yeast ratio 0.10%	Yeast ratio 0.15%	Yeast ratio 0.20%
1	1.11±0.01^{a}	1.14±0.01^{ab}	1.16±0.03^{ab}	1.17±0.01^{b}
2	1.15±0.02^{a}	1.19±0.03^{ab}	1.22±0.02^{ab}	1.24±0.03^{b}
3	1.18±0.01^{a}	1.23±0.01^{ab}	1.25±0.01^{ab}	1.27±0.02^{b}
4	1.20±0.03^{a}	1.27±0.02^{ab}	1.30±0.00^{ab}	1.32±0.02^{b}
5	1.23±0.01^{a}	1.29±0.04^{ab}	1.34±0.02^{ab}	1.39±0.00^{b}
6	1.28±0.02^{a}	1.34±0.02^{ab}	1.38±0.03^{ab}	1.42±0.01^{b}
7	1.31±0.00^{a}	1.40±0.01^{ab}	1.44±0.00^{ab}	1.55±0.03^{b}
8	1.35±0.02^{a}	1.42±0.00^{ab}	1.49±0.01^{ab}	1.68±0.02^{b}
9	1.37±0.03^{a}	1.46±0.01^{ab}	1.53±0.03^{ab}	1.71±0.01^{b}

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 11. Effect of yeast ratio to sensory characteristics in wort

Fermentation time (days)	Sensory score of wort by different yeast ratio			
	Yeast ratio 0.05%	Yeast ratio 0.10%	Yeast ratio 0.15%	Yeast ratio 0.20%
1	2.78±0.01^{a}	2.94±0.02^{ab}	3.11±0.00^{ab}	3.15±0.01^{b}
2	2.84±0.00^{a}	3.01±0.00^{ab}	3.18±0.01^{ab}	3.20±0.03^{b}
3	2.98±0.03^{a}	3.06±0.01^{ab}	3.25±0.02^{ab}	3.28±0.01^{b}
4	3.12±0.00^{a}	3.23±0.03^{ab}	3.36±0.01^{ab}	3.40±0.02^{b}
5	3.17±0.01^{a}	3.44±0.00^{ab}	3.59±0.02^{ab}	3.62±0.01^{b}
6	3.78±0.02^{a}	3.95±0.01^{ab}	4.04±0.03^{ab}	4.09±0.00^{b}
7	3.92±0.03^{a}	4.12±0.02^{ab}	4.47±0.01^{ab}	4.50±0.02^{b}
8	4.13±0.01^{a}	4.38±0.03^{ab}	4.53±0.02^{ab}	4.57±0.03^{b}
9	4.19±0.03^{a}	4.41±0.00^{ab}	4.62±0.00^{ab}	4.65±0.00^{b}

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 12. Effect of the sencondary fermentation to wine quality

Criteria	Secondary fermentation (weeks)			
	1	2	3	4
Soluble dry matter (^{o}Brix)	10.03±0.02^{a}	9.82±0.03^{ab}	9.66±0.02^{ab}	9.51±0.02^{b}
Ethanol (%v/v)	7.45±0.03^{b}	7.61±0.02^{ab}	7.84±0.03^{ab}	7.90±0.01^{a}
Acidity (g/l)	1.53±0.03^{b}	1.54±0.00^{ab}	1.54±0.01^{ab}	1.56±0.00^{a}
Sensory score	4.62±0.00^{b}	4.74±0.03^{ab}	4.80±0.00^{a}	4.80±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%).

Two different strains of *Saccharomyces cerevisiae* NCIM 3095 and NCIM 3287 were evaluated in the production of guava fruit wine. Guava must concentrations were adjusted to 22°Brix with sucrose solution, and batch fermentations were performed. For guava wine production *Saccharomyces cerevisiae* NCIM 3095 gave much better results as compare to *Saccharomyces cerevisiae* NCIM 3287 (Sevda SB and Rodrigues L, 2011). A study was conducted to produce wine from juice of guava (*Psidium guajava* L.) cv. Punjab Pink following treatment with pectinase enzyme. Ethanolic fermentation of both pectinase treated and untreated guava juice was optimized at 25°Brix (Pooja Nikhanj et al., 2017)

3.3 Effect of yeast inculate for wort fermentation

Guava juice after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.05, 0.1, 0.15, 0.20%). After 9 days of fermentation at 11.5^{o}C, we noticed the change of soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), and sensory

characteristics (score) in wort as in table 8, 9, 10 and 11. We found that the approprate yeast inculate should be 0.15% to get the highest wort quality.

Production of wine from over ripe guava (*Psidium guajava*) and ber (*Ziziphus mauritiana*) fruits using *Saccharomyces crevices* was conducted. The juices were adjusted with different TSS as 10, 15, 20, 25 and 30% by adding cane sugar in powder form and samples were fermented at 30 °C by using *Saccharomyces cerevicesvar*. It was seen that juice having TSS 15% showed higher ethanol production as compare to juices having different TSS in both guava and ber fruit juices. It was shown that ber and guava juices having pH 4 yield higher alcohol as compare to samples having different pH (Kaiser Younis et al., 2014).

A study was conducted to produce wine from juice of guava (*Psidium guajava* L.) cv. Punjab Pink following treatment with pectinase enzyme. Ethanolic fermentation of both pectinase treated and untreated guava juice was optimized at 25°C with 9% (v/v) inoculum size of *Saccharomyces cerevisiae* MTCC 11815 and 0.3% (w/v) DAHP supplementation under laboratory conditions. Ethanolic fermentation of treated guava 'must' resulted in 13.2 + 0.15 % (v/v) ethanol production in 8 days with an ethanol yield of 0.492g/g, while untreated juice resulted in 13.0 + 0.04 % (v/v) ethanol production with an ethanol yield of 0.487 g/g (Pooja Nikhanj et al., 2017).

3.4 Effect of secondary fermentation to wine quality

We preserved guava wine at 8.5^{o}C in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored residual soluble dry matted (^{o}Brix), ethanol (% v/v), acidity (g/l), and sensory characteristics (score) in wine. Our results were elaborated in table 12. 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.

A study was conducted to produce wine from juice of guava (*Psidium guajava* L.) cv. Punjab Pink following treatment with pectinase enzyme. Sensory analysis of guava-wine revealed that wine prepared from untreated guava 'must' was of standard quality as compared to superior quality of wine prepared from treated one at 30 days of storage. However, both wines were superior as reflected by sensory scores of 68.1 ± 1.22 and 70.3 ± 1.22, respectively, at 90 days of storage (Pooja Nikhanj et al., 2017).

4. CONCLUSION

Guava (*Psidium guajava* L.) is a native fruit of the American tropics with commercial applications for its taste, flavor and aroma. It is a fruit with good nutritional attributes but has short shelf-life under the prevailing weather conditions in tropical countries. Mature and ripe guavaes with their high composition of fermentable reducing sugars such as glucose, sucrose and fructose could serve as substrates for fruit wine production using wine yeast (*Saccharomyces cerevisiae*), thus transforming a perishable products to more stable and value added product. Therefore, production of wine from this fruit can help increase wine variety and reduce post-harvest losses.

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