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Utilization of Straw Berry (*Muntingia Calabura*) Fruit for Wine Fermentation

Nguyen Phuoc Minh[1,*], Nguyen Phu Thuong Nhan[2], Lam Binh Thuong[3], Bui Ha Thanh[4], Nguyen Thi Tho[5], Ly Xuan Thien[6]

[1]*Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh, Vietnam*
[2]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[3]*Can Tho University, Can Tho City, Vietnam*
[4]*Vinh Long Education Technology, Vinh Long Province, Vietnam*
[5]*Ho Chi Minh University of Technology, Ho Chi Minh City, Vietnam*
[6]*Tien Giang University, Tien Giang Province, Vietnam*

Abstract.

Straw berry (*Muntingia calabura)* is an underexploited fruit in South of Vietnam. Muntingia calabura L is a fast growing medicinal plant, attracts fruit eating birds such as flower peckers. It is a sweet fruit and mainly used as fresh fruit consumption. *Muntingia calabura* fruit is rich in antioxidant polyphenol and possess significant antioxidant activity, is an important and interesting finding. There is limited study mentioning to processing of this nutritional fruit. Therefore we explored a wine fermentation from this fruit 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 0.15% pectinase was used for juice extraction in 20 minutes, 0.3% *sacchromyces cerevisiae* was used for the main fermentation at 11.5^{o}C in 9 days, and 4 weeks of sencondary fermentation in dark bottle at 9.5^{o}C was applied to get a pleasant *Muntingia calabura* quality.

Keywords: *Muntingia calabura, wine, fermentation, sacchromyces cerevisiae, pectinase*

1. Introduction

Straw berry (*Muntingia calabura)* belonging to Elaeocarpaceae family. This plant is a fast-growing tree of slender proportions, reaching a height of approximately 7.5–12 m with nearly horizontal spreading branches. It is often seen growing as roadside trees (Ragragio EM et al., 2014), also used as an air pollution tolerance indicator. It is an annual plant, flowers throughout the year. *M. calabura* are evergreen approximately 5–12.5 cm long, alternate lanceolate or oblong, long pointed at the apex, oblique at the base with dark green color and minutely hairy on the upper surface, gray- or brown-hairy on the underside and irregularly toothed. The leaves are rich in flavanoidal compounds like flavones, flavanones, flavans, and biflavans as the major constituents, possessing antidiabetic and cytotoxic activities (Perez GRM et al., 1998; Nshimo CM et al., 1993). Straww cherry (*Muntingia calabura*) leaf extract contains flavanoid compounds, terpenoids, tannins and high antioxidant activity (Diana Triswaningsih et al., 2017). Its leaves are distinctively lanceolate in shape, with margins irregularly serrate and fruits are berries which turn red on maturation and are sweet in taste (Bayer C et al., 1998). The fruits are abundant, in round shape; approximately 1–1.25 cm wide, with red or yellow, thin, smooth, tender skin and light-brown, soft, juicy pulp, with very sweet, musky, fig-like flavor, and filled with exceedingly tiny, yellowish seeds (N. D. Mahmood et al., 2014). Phytochemical studies revealed that it presented phenolics, flavonoids, terpenoids, reducing sugars, saponins, tannins, and carbohydrates, anthocyanins, ascorbic acid and vitamin E. (Preethi Kathirvel, 2017; Vijayanand S and Ann Steffy Thomas, 2018). Their fruits are processed in to jams, leaves are used for making tea. Fruit from Muntingia is also harvested for export overseas. (Marimuthu Krishnaveni and Ravi Dhanalakshmi, 2014). Reports are there for its anti-tumor, antinociceptive, anti-inflammatory, anti-pyretic, antibacterial, antiproliferative and antioxidant, antihypertensive, antiulcer and antistaphyloccocal activities (Kaneda NJM et al., 1991; Su BNE et al., 2003; Zakaria ZA et al., 2007; Shih CD et al., 2006; Preethi, Kathirvel et al., 2012; Consolacion Y. Ragasa et al., 2015). The incorporation of fruits into routine diet could prevent the risk of cardiovascular diseases, ageing, inflammations and cancers due to antioxidant compounds present in the fruits (Preethi Kathirvel, 2017).

Muntingia calabura is an underutilized fruit crop and still now there is very limited research available Enhancement of antioxidant profile of Japanese cherry (*Muntingia calabura* Linn.) by alcoholic fermentation regarding to processing of this fruit into value added product was examined (A.P Ananda et al., 2012). There was a research examined production of bioethanol from *Muntingia calabura* (Ravishankar .B .V et al., 2016). *Muntingia calabura* was used as raw material to produce bio-ethanol by using *Saccharomyces cerevisiae* (Ravishankar B. V. et al., 2017). In order to improve the added value of this fruit, 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 *Muntingia calabura* in Ben Tre province, Vietnam. They must be harvested from gardens 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 *Muntingia calabura*, 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.

Figure 1. *Muntingia calabura*

2.2 Research method

2.2.1 Effect of pectinase concentration and time for juice extraction

Muntingia calabura extract was treated with pectinase enzyme with different concentration (0.05, 0.10, 0.15, 0.20%) in different duration (10, 15, 20, 25 minutes). We analyzed the extract recovery (%), viscosity (cP) and turbidity (mJ/cm^2).

2.2.2 Effect of yeast inculate for wine fermentation

Muntingia calabura wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.1, 0.2, 0.3, 0.4%). After 9 days of fermentation at 11.5^{o}C, we analyzed the soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), and sensory characteristics (score) in wine.

2.2.3 Effect of secondary fermentation to wine quality

We preserved *Muntingia calabura* 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.

2.2.4 Statistical analysis

The experiments were performed in triplicate. Statistical analysis was conducted by the Statgraphics Centurion XVI.

3. Result & Discussion

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

Muntingia calabura extract was treated with pectinase enzyme with different concentration (0.05, 0.10, 0.15, 0.20%) in different duration (10, 15, 20, 25 minutes). Our results were depicted in table 1, 2 and 3. We clearly found that 0.15% pectinase in 30 minutes treatment was optimal for *Muntingia calabura* 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 (%)**			
	10 minutes	15 minutes	20 minutes	25 minutes
0.05	62.49±0.02^{b}	62.67±0.02^{b}	62.96±0.00^{b}	62.98±0.02^{b}
0.10	62.65±0.01^{ab}	62.81±0.01^{ab}	63.01±0.01^{ab}	63.04±0.03^{ab}
0.15	62.89±0.03^{a}	62.97±0.01^{a}	63.14±0.03^{a}	63.15±0.01^{a}
0.20	62.91±0.00^{a}	63.02±0.00^{a}	63.16±0.01^{a}	63.17±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 2. Viscosity (cP) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Viscosity (cP)			
	10 minutes	15 minutes	20 minutes	25 minutes
0.05	1.19±0.02^{a}	1.15±0.04^{a}	1.11±0.02^{a}	1.09±0.01^{a}
0.10	1.12±0.03^{ab}	1.09±0.00^{ab}	1.07±0.01^{ab}	1.05±0.00^{ab}
0.15	1.05±0.02^{b}	1.03±0.03^{b}	1.01±0.01^{b}	0.99±0.01^{b}
0.20	1.04±0.01^{b}	1.02±0.01^{b}	1.00±0.00^{b}	0.98±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 3. Turbidity (mJ/cm^2) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Optical density (mJ/cm^2)			
	10 minutes	15 minutes	20 minutes	25 minutes
0.05	72.25±0.02^{a}	72.19±0.01^{a}	72.05±0.01^{a}	72.02±0.02^{a}
0.10	71.89±0.01^{ab}	71.84±0.02^{ab}	71.80±0.01^{ab}	71.78±0.00^{ab}
0.15	71.42±0.02^{b}	71.38±0.01^{b}	71.31±0.03^{b}	71.29±0.03^{b}
0.20	71.39±0.03^{b}	71.35±0.03^{b}	71.29±0.02^{b}	71.25±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 4. Effect of yeast ratio to soluble dry matter (^{o}Brix) in wine

Fermentation time (days)	**Soluble dry matter in wine (oBrix)**			
	Yeast ratio 0.1%	Yeast ratio 0.2%	Yeast ratio 0.3%	Yeast ratio 0.4%
1	22.79±0.02^{a}	21.64±0.02^{a}	20.04±0.03^{a}	19.98±0.01^{a}
2	19.99±0.01^{ab}	19.60±0.01^{ab}	18.41±0.02^{ab}	18.35±0.02^{ab}
3	18.48±0.00^{b}	18.20±0.01^{b}	17.35±0.01^{b}	17.22±0.01^{b}
4	16.59±0.00^{bc}	16.18±0.01^{bc}	15.89±0.03^{bc}	15.80±0.03^{bc}
5	14.78±0.02^{c}	14.31±0.03^{c}	13.92±0.03^{c}	13.88±0.01^{c}
6	13.44±0.01^{cd}	13.18±0.03^{cd}	12.83±0.01^{cd}	12.75±0.02^{cd}
7	12.28±0.03^{d}	12.05±0.02^{d}	11.94±0.02^{d}	11.87±0.01^{d}
8	11.40±0.03^{de}	11.12±0.02^{de}	10.85±0.02^{de}	10.69±0.01^{de}
9	11.03±0.03^{e}	10.98±0.02^{e}	10.66±0.02^{e}	10.60±0.01^{e}

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 0.1%	Yeast ratio 0.2%	Yeast ratio 0.3%	Yeast ratio 0.4%
1	1.45±0.01^{e}	1.60±0.01^{e}	2.24±0.02^{e}	2.30±0.02^{e}
2	1.89±0.03^{de}	1.13±0.02^{de}	2.42±0.01^{de}	2.71±0.01^{de}
3	2.43±0.02^{d}	2.75±0.03^{d}	2.96±0.02^{d}	3.11±0.03^{d}
4	2.78±0.01^{cd}	2.98±0.03^{cd}	3.35±0.03^{cd}	3.43±0.01^{cd}
5	3.03±0.02^{c}	3.25±0.01^{c}	3.66±0.01^{c}	3.75±0.01^{c}
6	3.36±0.01^{bc}	3.47±0.02^{bc}	3.95±0.01^{bc}	3.99±0.02^{bc}
7	3.90±0.03^{b}	4.20±0.01^{b}	4.48±0.02^{b}	4.50±0.02^{b}
8	4.29±0.03^{ab}	4.48±0.01^{ab}	4.86±0.02^{ab}	4.92±0.02^{ab}
9	4.41±0.03^{a}	4.54±0.01^{a}	4.95±0.02^{a}	5.00±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 6. Effect of yeast ratio to acidity (g/l) in wine

Fermentation time (days)	Acidity in wine (g/l)			
	Yeast ratio 0.1%	Yeast ratio 0.2%	Yeast ratio 0.3%	Yeast ratio 0.4%
1	1.06±0.01^{e}	1.11±0.02^{e}	1.20±0.02^{e}	1.23±0.01^{e}
2	1.11±0.03^{de}	1.18±0.03^{de}	1.29±0.01^{de}	1.31±0.02^{de}
3	1.35±0.01^{d}	1.42±0.01^{d}	1.48±0.02^{d}	1.52±0.01^{d}
4	1.66±0.02^{cd}	1.75±0.02^{cd}	1.82±0.01^{cd}	1.84±0.03^{cd}
5	1.99±0.01^{c}	2.01±0.01^{c}	2.06±0.00^{c}	2.07±0.02^{c}
6	2.04±0.01^{bc}	2.10±0.01^{bc}	2.17±0.01^{bc}	2.20±0.01^{bc}
7	2.11±0.02^{b}	2.18±0.03^{b}	2.30±0.03^{b}	2.34±0.00^{b}
8	2.17±0.02^{ab}	2.25±0.03^{ab}	2.41±0.03^{ab}	2.43±0.00^{ab}
9	2.20±0.02^{a}	2.29±0.03^{a}	2.47±0.03^{a}	2.50±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 7. Effect of yeast ratio to soluble dry sensory characteristics (score, 1-5) in wine

Fermentation time (days)	Sensory score of wine (1-5) by different yeast ratio			
	Yeast ratio 0.1%	Yeast ratio 0.2%	Yeast ratio 0.3%	Yeast ratio 0.4%
1	1.41±0.01^{d}	1.98±0.01^{d}	2.05±0.01^{d}	2.20±0.01^{d}
2	1.46±0.02^{cd}	2.05±0.00^{cd}	2.27±0.02^{cd}	2.34±0.02^{cd}
3	1.90±0.01^{c}	2.11±0.01^{c}	2.30±0.03^{c}	2.41±0.00^{c}
4	2.11±0.03^{bc}	2.34±0.02^{bc}	2.54±0.00^{bc}	2.61±0.01^{bc}
5	2.54±0.00^{b}	2.65±0.01^{b}	2.78±0.00^{b}	2.84±0.02^{b}
6	3.03±0.02^{ab}	3.08±0.00^{ab}	3.17±0.01^{ab}	3.20±0.01^{ab}
7	3.09±0.01^{a}	3.17±0.03^{a}	3.36±0.02^{a}	3.40±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 8. Effect of the sencondary fermentation to wine quality

Criteria	Secondary fermentation (weeks)			
	1	2	3	4
Soluble dry matter (^{o}Brix)	10.50±0.01^{a}	10.44±0.01^{ab}	10.19±0.01^{ab}	10.00±0.02^{b}
Ethanol (%v/v)	5.10±0.02^{b}	5.17±0.02^{ab}	5.25±0.02^{ab}	5.37±0.02^{a}
Acidity (g/l)	2.56±0.03^{b}	2.59±0.00^{ab}	2.60±0.00^{ab}	2.61±0.00^{a}
Sensory score	3.40±0.02^{b}	3.51±0.01^{ab}	3.67±0.01^{ab}	3.70±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%).

3.2 Effect of yeast inculate for wine fermentation

Muntingia calabura wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.1, 0.2, 0.3, 0.4%). 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 wine as in table 4, 5, 6 and 7. We found that the approprate yeast inculate should be 3% to get the highest wine quality.

Straw cherry (*Muntingia calabura)* with supplements to promote yeast growth was subjected to alcoholic fermentation for 28 days. A clear fluid with 10 percent alcohol (v/v) was obtained. It contained 1.68 mg per ml total phenolics (as gallic acid equivalent) and 1.3 mg per ml of reducing power factor (as tannic acid equivalent) which were about 2 fold and 1.6 fold increases respectively over the unfermented must. The wine of straw cherry (*Muntingia calabura*) was found to be comparable in acceptability to a market sample of traditional white wine (A.P Ananda et al., 2012).

Muntingia calabura was used as raw material to produce bio-ethanol by using *Saccharomyces cerevisiae.* The results obtained from this work shows that the higher rate of ethanol production through fermentation at the operating conditions (pH 5.5, temperature 30±2°C, speed 80 rpm, fermentation period 15 days) were feasible (Ravishankar B. V. et al., 2017).

3.3 Effect of secondary fermentation to wine quality

We preserved *Muntingia calabura* wine at 9.5°C in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored soluble dry matted (°Brix), ethanol (% v/v), acidity (g/l), and sensory characteristics (score) in wine. Our results were elaborated in table 8. 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.

The utility of less utilized fruits as a possible source of ethanol production in a process without aeration was investigated by using fruits of Muntingia calabura. The fruit juice was subjected to fermentation by *Saccharomyces cerevisiae*. The amount of ethanol produced after fermentation was analyzed by gas chromatography and the ethanol production is almost equal to any commercial fruit ethanol production (Ravishankar .B .V et al., 2016).

4. Conclusion

Muntingia calabura contains different phytochemicals that include terpenoids, reducing sugars, flavonoids, saponins, tannins, phenols and carbohydrates. Its fruit possess a potent antioxidant activity. We have successfully utilized *Muntingia calabura* 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. These results were important because they could help wine makers to arrange proper processing method and storage.

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