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Wine production from ripen pond apple (*Annona glabra* L.) fruit

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Abstract

Pond apple (*Annona glabra* L.) trees were widely distributed in swamp regions of Mekong Delta, Vietnam. Pond apple fruits turned from green to yellow when ripening. Ripen pond apple fruits contained numerous phenolic constituents with valuable phytochemical benefits. However, ripen pond apple fruits were not successfully utilized as other commercial fruits. This research examined the possibility of wine production utilized from ripen pond apple fruits. Different various technical variables of fermentation affecting to the quality of pond apple wine were thoroughly examined. Ripen pond apple fruits were naturally collected from Soc Trang province, Vietnam. Ripen pond apple fruits were peeled, blended, deseeded, crushed, enzyme-treated (pectinase 25 mg/l), added with sugar (5-13% w/w), pasteurized (sulphite 30 mg/l), inoculated with yeast *Saccharomyces pastorianus* ratio (0.1-0.5%), macerated temperature (14-22 °C) in different time (6-14 days). Malolactic fermentation was performed in anaerobic condition at 12 °C in different durations (4-20 weeks). At the end of malolactic fermentation, wine was racked and clarified with different fining agents (bentonite, polyvinylpyrrolidone, wheat gluten, gelatin, kaolin) at 0.03% (v/v). Results showed that must should be added with 9% sugar and 0.4% yeast inoculation, fermentation temperature of 16 °C in 10 days. Malolactic fermentation could be terminated at 12 weeks. Gelatin revealed the best candidate among different clarifying agents to remove turbidity in pond apple wine while retaining the most total phenolic content and antioxidant capacity. Under above technical variable conditions, fermentation gave the high ethanol content (4.26±0.02 % v/v); the total phenolic content (32.79±0.00 mg GAE/100 ml), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging (11.84±0.01 %), overall acceptance (8.34±0.01 score) meanwhile low turbidity (24.41±0.00 NTU) was also noticed. High ethanol content and phytochemical retention contributed to the high sensory score of pond apple wine. These quality parameters were acceptable for an alcoholic drink. Ripen pond apple fruit would be a promising carbohydrate source to convert into a new fruit wine with a pleasant alcoholic flavor and attractive appearance.

Keywords

clarification, maceration, malolactic fermentation, pond apple wine

Introduction

Pond apple (*Annona glabra* L.) belonged to a member of the custard apple family, (Annonaceae). Other species included *Annona cherimola* Mill, cherimoya, *A. muricata* L., soursop, *A. reticulata* L., custard apple and *A. squamosa* L., sugar

apple. Pond apple was a tropical tree distributed widely in Vietnam and other Asian countries. It was easily vulnerable to low-iron stress but well adapted in high-salinity or dry condition of coastal landscapes. Pond apple created ideal shelter for numerous wildlife habitat, feed for different species, protection from soil erosion, and nutrient balance in ecosystem. This fruit was edible and similar to a custard apple. The ripen fruit could be eaten, boiled, converted into jam and jelly (1). Pond apple fruit contained numerous phytochemical constituents such as peptides, acetogenins, alkaloids, *ent*-kauranes (2-5). Different therapeutic benefits were reported (6).

Pond apple fruit normally dropped down when ripening. Ripen pond apple fruit was highly perishable and impossible to be stored long consumption, thus leading fruit wastage. Fermentation was one of versatile techniques to convert overripe fruits into biomaterials, like wine, vinegars, bioethanol, organic acids and supplements, which minimized fruit wastage and enhanced economic values of crops (7, 8). There was no any research mentioned to the utilization of ripen pond apple fruit for wine production. Purpose of the present research examined various technical variables such as sugar addition, yeast ratio, maceration temperature, maceration time, malolactic fermentation time and clarifying agents to the physicochemical, phenolic, antioxidant and sensory attributes of pond apple wine.

Materials and Methods

Material

Ripen pond apple fruits were naturally harvested in Soc Trang province, Vietnam. After being collected, they were stored in dry cool place and transferred to laboratory as quickly as possible. Starter culture containing approximately 9 log cfu/g of *Saccharomyces pastorianus* was supplied from Vinabeco (Vietnam). *Pectinex*® Ultra SP-L was purchased from Novozymes. Refine sugar was purchased from grocery store. Chemical reagents such as ethanol, acetonitrile, Folin-Ciocalteu phenol, trichloro-acetic acid, DPPH (2, 2-Diphenyl picrylhydrazyl) reagent, gallic acid, Na_2CO_3,, phenolphthalein were all analytical grade supplying from Sigma Aldrich (Steinheim, Germany) and Fluka (USA). Polyvinylpolypyrrolidone (PVPP) was purchased from Ludwigshafen (Germany). Bentonite was obtained from Perdomini (Verona, Italy). wheat gluten was received from Foodchem International Corporation (China). Gelatin was supplied from Henan E-King Gelatin Co.Ltd (China). Kaolin was purchased from Nong Thanh Viet Co. Ltd (Vietnam).

Researching method

Ripen pond apple fruits were peeled by stainless spoon to remove skin. Peeled pond apple pulp was then pulverized by blending. The obtained homogenous must was then deseeded, crushed, enzyme-treated (pectinase 25 mg/l), added with sugar (5-13% w/w), pasteurized (sulphite 30 mg/l), inoculated with yeast ratio (0.1-0.5%). The maceration was conducted in different time (6-14 days) and temperature (14-22 °C) with daily aeration by stirring twice to promote yeast multiplication. The must was sieved to separate the crushed fruits. The collected filtrate was transferred to malolactic fermentation in anaerobic condition at 12 °C in different time (4-20 weeks). The malolactic fermentation was terminated and wine was racked. To avoid turbidity for wine, clarification was needed. Amount of 10 g of each fining agent (bentonite, polyvinylpyrrolidone, wheat gluten, gelatin, kaolin) was dispensed in 500 ml of hot water for 3 min to create slurry. They were then assessed individually by adding 30 ml slurry of each fining agent into 1 l of wine. The flocculation in wine was removed by filtration and wine was ready for analysis.

Determination of physicochemical quality, phenolic content and antioxidant capacity

Residual sugar (g/l) was determined using Clinitest® (9). Ethanol (% v/v) was examined by capillary gas chromatography using megapore polar column (10). Total phenolic content (mg GAE/100 g) was examined by Folin-Ciocalteu reagent protocol (11). Extract was disolved with 90% ethanol (v/v) in a 10 ml tube and centrifuged at 4000 g within 2.5 min. A 1.4 ml of the extract was combined with 2.0 ml Folin-Ciocalteu reagent 10% (w/v). After 10 min of reaction, 4.0 ml of Na_2CO_3 (5% w/v) was added. Reaction lasted for one hour without light, the absorbance was recorded at 760 nm by spectrophotometer (model: UV-1800, Shimazu, Japan) and compared with a pure linear of gallic acid (0-300 mg/l). R^2 of the calibration curve was noticed at 0.98. DPPH free radical scavenging (%) was estimated using UV-VIS spectrophotometer (model: model: UV-1800, Shimazu, Japan) with mobile phase methanol and water mixed online in the ratio of 80:20 (v/v), injected at a current speed of 1.0 ml/min. Aliquots of the samples 0.5 ml were supplemented with 3.5 ml of the 0.05 mM DPPH solution in the dark place, and the mixture was thoroughly vibrated and then incubated for 20 min at 37 °C. DPPH peaks were quantified at wavelength 517 nm (Andriana et al. 2019). R^2 of the calibration curve was noticed at 0.95. Turbidity (NTU or Nephelometric Turbidity Units) was evaluated by nephelometric turbidimeter (model: TB 250 WL, Lovibond, Thomas Scientific, USA). Overall acceptance (sensory score) was determined by a group of 11 specialists using 9-point Hedonic scale. Fifteen trained specialists were at the age 28-30 years old. They evaluated samples based on color, flavor and taste which were expressed as overall acceptance on scale 1-9.

Statistical analysis

The experiments were prepared in triplicate with various sets of samples. The data were presented as mean ± standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI. The mean value ($\bar{x}$) and standard deviation (2*s*) of a set of data were obtained by analysis of random samples estimating the population statistics. 95% of results would be expected to lie within the range . The lower and upper bounds of this range were described at the 95% confidence limits of the results. The differences between the treated samples were analyzed using a one-way analysis of variance (ANOVA). A significant

value is set at a 95% confidence interval (p 0.05). If significant differences were found, then post hoc analysis was performed using Duncan's multiple range tests. .

Results and Discussion

Effect of sugar supplementation in the maceration

Sugar addition showed a significant difference on residual sugar, ethanol, total phenolic content and DPPH free radical scavenging during maceration (Table 1). Supplementation of 9% sugar into pond apple must induced the highest ethanol (2.42±0.00 % v/v), total phenolic content (48.60±0.15 mg GAE/100 ml) and DPPH free radical scavenging (20.51±0.03 %) while maintaining a medium amount of residual sugar (3.19±0.03 g/l). Hence, sugar addition was fixed at 9% for next experiments. The initial sugar content played important role in the fermentation process. It affected not only the fermentation kinetics and ethanol formation but also the overall metabolite-profile (12, 13). The proper total soluble solid content in must was very important to impair the capability of yeast to multiply during exponential growth, because there was enough available nitrogen in must for yeast proliferation (14). However, condensed sugar content in must resulted to negative effect with reduction of yeast cell size, cell division, viable cell density. The concentrated dry matter in must conjured the yeast high-osmolarity glycerol response to hyperosmotic condition (14).

Table 1. Effect of sugar addition (%) to residual sugar (g/l), ethanol (%v/v), total phenolic content (mg GAE/100 ml), DPPH free radical scavenging (%) in 6 days of maceration at 14 °C with 0.1% yeast

Parameters	Sugar addition (%)				
	5	7	9	11	13
Residual sugar (g/l)	2.47±0.02^{c}	2.83±0.01^{bc}	3.19±0.03^{b}	3.72±0.02^{ab}	4.46±0.00^{a}
Ethanol (% v/v)	0.70±0.01^{d}	1.25±0.02^{c}	2.42±0.00^{a}	1.59±0.03^{b}	1.43±0.02^{bc}
Total phenolic content (mg GAE/100 ml)	38.25±0.14^{c}	40.38±0.11^{bc}	48.60±0.15^{a}	45.79±0.17^{ab}	43.27±0.12^{b}
DPPH free radical scavenging (%)	12.04±0.05^{c}	14.63±0.02^{bc}	20.51±0.03^{a}	18.06±0.01^{ab}	16.42±0.02^{b}

Values are the mean of three replications; values in row followed by the same superscript lowercase letter/s are not differed significantly (α = P=0.05) by Duncan's multiple range test

Effect of yeast ratio in maceration

Yeast ratio played an important role in maceration. When increasing yeast ratio (0.1-0.5%), residual sugar, total phenolic content and DPPH free radical scavenging decreased gradually; meanwhile ethanol content increased. There was no significant difference of residual sugar, ethanol content, total phenolic content and DPPH free radical scavenging by 0.4% and 0.5% yeast in inoculation (Table 2). To save production cost, we selected 0.4% yeast for further experiments. *Saccharomyces pastorianus* was a bottom fermenting yeast, adaptable to low temperatures, flocculated well, deposited fast and metabolized glucose, fructose and complex sugars as maltose and maltotriose (15). *S. pastorianus* provided higher amount of rose like aromas in the wine (16). The wines fermented with *S. pastorianus* produced significant lower acetic acid and greater malic acid degradation compared to the wines fermented with *S. cerevisiae* (17). Incorporation of *S. pastorianus* and *S. bayanus* provided wine with a stronger intensity of citrus flavor than the wine fermented with the pure *S. cerevisiae* (17). A higher yeast inoculum rate was desirable to obtain the required ethanol content in reasonable fermentation duration. Increasing yeast inoculation level also troubleshoot the sluggish fermentation caused by poor nitrogen source in high-gravity must during alcoholic fermentation (18). The lower yeast inoculation ratio induced an insufficient amount of sugar metabolized to release the desirable ethanol content. Moreover, the lower inoculation level also converted a higher percentage of sugar to acetic acid and glycerol compared to the higher inoculation ratio. Proper yeast inoculation rate directly influenced the number of viable cells during fermentation and hence impacted to the time needed for a complete fermentation (14).

Table 2. Effect of yeast ratio (%) to residual sugar (g/l), ethanol (%v/v), total phenolic content (mg GAE/100 ml), DPPH free radical scavenging (%) in 6 days of maceration at 14 °C with 9% sugar addition

Parameters	Yeast ratio (%)				
	0.1	0.2	0.3	0.4	0.5
Residual sugar (g/l)	3.19±0.03^{a}	2.10±0.04^{b}	1.78±0.01^{bc}	1.25±0.03^{c}	1.16±0.02^{c}
Ethanol (% v/v)	2.42±0.00^{c}	2.75±0.02^{bc}	3.01±0.03^{b}	3.29±0.00^{ab}	3.60±0.01^{a}
Total phenolic content (mg GAE/100 ml)	48.60±0.15^{a}	45.62±0.08^{ab}	43.19±0.11^{b}	40.85±0.13^{bc}	37.59±0.10^{c}
DPPH free radical scavenging (%)	20.51±0.03^{a}	19.07±0.00^{ab}	17.86±0.02^{b}	15.52±0.03^{bc}	13.90±0.01^{c}

Values are the mean of three replications; values in row followed by the same superscript lowercase letter/s are not differed significantly (α = P=0.05) by Duncan's multiple range test

Effect of fermentation temperature in maceration

Maceration temperature caused significant influence for yeast growth and biosynthesis of ethanol. There was no significant difference of residual sugar, ethanol content, total phenolic content, and DPPH free radical scavenging by 16 °C and 18 °C maceration. However, we could see that 16 °C was selected instead of 18 °C to maintain total phenolic content and DPPH free radical scavenging while achieving acceptable ethanol content (Table 3). The fermentation temperature strongly influenced the finished composition of wine (19, 20). Moreover, it also affected the

Table 3. Effect of maceration temperature (°C) to residual sugar (g/l), ethanol (%v/v), total phenolic content (mg GAE/100 ml), DPPH free radical scavenging (%) in 6 days with 9% sugar addition and 0.4% yeast

Parameters	Maceration temperature (°C)				
	14	16	18	20	22
Residual sugar (g/l)	1.25±0.03^{b}	1.02±0.01^{bc}	0.71±0.02^{c}	1.49±0.01^{ab}	1.70±0.03^{a}
Ethanol (% v/v)	3.29±0.00^{b}	3.60±0.03^{ab}	3.87±0.00^{a}	3.00±0.02^{bc}	2.64±0.00^{c}
Total phenolic content (mg GAE/100 ml)	40.85±0.13^{b}	38.26±0.09^{bc}	35.70±0.07^{c}	42.39±0.11^{ab}	45.17±0.13^{a}
DPPH free radical scavenging (%)	15.52±0.03^{b}	14.08±0.04^{bc}	12.63±0.05^{c}	15.98±0.02^{ab}	17.15±0.03^{a}

Values are the mean of three replications; values in row followed by the same superscript lowercase letter/s are not differed significantly (α = P=0.05) by Duncan's multiple range test

amount of biomass (21). Low temperature was commonly implemented to avoid the decomposition of aroma components (22, 23). The higher the temperature the lower the ethanol formed from the same initial degree of sugar (24). Therefore, winemakers preferred the fermention of white wines at lower temperatures to improve the formation and sustain of aroma volatiles as it improved the content of ethyl esters and acetates, and lowered ethanol content (25). Temperature impacted to the resistance of yeasts to ethanol content, growth speed, fermentation kinetic, cell viability, length of lag phase, enzyme and membrane feature (26). Temperature should be appropriate to create favorable condition for yeast viability (27). A fermentation temperature of lower than 35 °C facilitated for yeast to release more ethanol from fermentable sugars (28). *Saccharomyces pastorianus* showed efficient fermentation, high antioxidant activity, excellent flocculation capacity at 15 °C in the brewing industry (29). Inoculation with *S. pastorianus* at 13 °C induced the specific aromas of wine stronger than sample inoculated at 18 °C (17). An extended maceration time at high temperature over the last phase of the process would be desirable to achieve aromatic wine with the expected color durability (30).

Effect of fermentation time in maceration

Extending maceration time induced to the more ethanol content and the less residual sugar, total phenolic content and DPPH free radical scavenging. Maceration could be appropriately terminated at 10 days to obtain high ethanol content (3.98±0.03 % v/v) while retaining a fair content of total phenolic content (35.73±0.05 mg GAE/100 ml) and DPPH free radical scavenging (13.50±0.00 %) (Table 4). Yeast required enough time to adapt to substrate in the lag phase. Prolonged fermentation duration induced a higher content of ethanol as the starter culture would have enough time to biosynthesize alcohol dehydrogenase permitting the biotransformation of sugar to ethanol (31, 32). Soursop (*Annona muricata*) juice was converted into wine after 131 hrs of alcoholic fermentation at pH 4.99, and a 0.42 culture ratio (42:58, *P. pulmonarius mycelia*: *S. cerevisiae* (33). Extended fermentation time induced a reduction of total phenolic content in fermented rice (34). This was originated from the oxidative reactions by polyphenol oxidase during maceration (35). Total phenolic content decreased greatly in cider vinegar from raw juice (36). DPPH free radical scavenging of mulberry wine increased sharply in the first 2 days of fermentation, remained stable and decreased gradually until day 10th of fermentation (37).

Effect of malolactic fermentation time

Malolactic fermentation time contributed a great importance in aging process for wine. By prolonging malolactic fermentation time from 4-20 weeks, there were decelerating trends of residual sugar, total phenolic content and DPPH free radical scavenging. Meanwhile, ethanol content and overall acceptance went up by aging time. Malolactic fermentation time could be stopped at 12 weeks to maintain a trace amount of residual sugar (0.30±0.03 g/l), high ethanol content (4.28±0.02 % v/v), acceptable total phenolic content (33.54±0.04 mg GAE/100 ml), DPPH free radical scavenging (12.70±0.02%) and overall acceptance (7.69±0.04) (Table 5). High content of malic acid provided tart taste (38). During malolactic fermentation, decarboxylation process converted L-malic acid into L-lactic acid (39). Therefore, it caused a reduction of tart taste and increment of aroma complexity. Malolactic fermentation contributed a great improvement in sensory attributes of wine (40). Moreover, wine also had better microbial stability after malolactic fermentation (39). Malolactic fermentation induced minor impact on antioxidants (41). Through malolactic fermentation, peptidolytic and proteolytic activity broken down the proteins causing wine haze, supporting for wine clarification (42).

Table 4. Effect of maceration time (days) to residual sugar (g/l), ethanol (%v/v), total phenolic content (mg GAE/100 ml), DPPH free radical scavenging (%) in maceration temperature 16 °C with 9% sugar addition and 0.4% yeast

Parameters	Maceration time (days)				
	6	8	10	12	14
Residual sugar (g/l)	1.02±0.01^{a}	0.73±0.00^{ab}	0.64±0.01^{ab}	0.40±0.03^{b}	0.38±0.02^{b}
Ethanol (% v/v)	3.60±0.03^{b}	3.85±0.02^{ab}	3.98±0.03^{ab}	4.15±0.00^{a}	4.19±0.01^{a}
Total phenolic content (mg GAE/100 ml)	38.26±0.09^{a}	36.40±0.04^{ab}	35.73±0.05^{ab}	32.46±0.03^{b}	32.19±0.03^{b}
DPPH free radical scavenging (%)	14.08±0.04^{a}	13.75±0.02^{ab}	13.50±0.00^{ab}	10.28±0.01^{b}	10.03±0.02^{b}

Values are the mean of three replications; values in row followed by the same superscript lowercase letter/s are not differed significantly (α = P=0.05) by Duncan's multiple range test

Table 5. Effect of aging time (weeks) to residual sugar (g/l), ethanol (%v/v), total phenolic content (mg GAE/100 ml), DPPH free radical scavenging (%) and overall acceptance of wine in malolactic fermentation at 12 °C

Parameters	Malolactic fermentation time (weeks)				
	4	8	12	16	20
Residual sugar (g/l)	0.43±0.03^{a}	0.39±0.01^{ab}	0.30±0.03^{ab}	0.22±0.00^{b}	0.19±0.02^{b}
Ethanol (% v/v)	4.09±0.00^{b}	4.17±0.03^{ab}	4.28±0.02^{ab}	4.40±0.01^{a}	4.45±0.03^{a}
Total phenolic content (mg GAE/100 ml)	34.90±0.07^{a}	34.18±0.06^{ab}	33.54±0.04^{ab}	32.98±0.02^{b}	32.67±0.05^{b}
DPPH free radical scavenging (%)	13.07±0.03^{a}	12.85±0.04^{ab}	12.70±0.02^{ab}	12.25±0.03^{b}	12.18±0.01^{b}
Overall acceptance (sensory score)	5.89±0.02^{c}	7.25±0.01^{b}	7.69±0.04^{ab}	8.01±0.00^{a}	8.07±0.03^{a}

Values are the mean of three replications; values in row followed by the same superscript lowercase letter/s are not differed significantly (α = P=0.05) by Duncan's multiple range test

Effect of clarifying agent

Effect of different clarifying agents on turbidity, ethanol content, total phenolic, DPPH free radical scavenging, and overall acceptance of wine was presented in Table 6. There was no significant difference of ethanol content during clarification of wine among clarifying agents. It's obviously noticed that there was no significant difference of turbidity, ethanol content, total phenolic, DPPH free radical scavenging, and overall acceptance of wine by clarification gents of either gelatin or polyvinylpyrrolidone. However, gelatin revealed better retention of total phenolic, DPPH free radical scavenging, and overall acceptance of wine. Therefore, gelatin was selected for pond apple wine fermentation. Turbidity caused a less chance to deposit due to the fine particle and soluble pectins, which accelerated the viscosity of wine (43). Colloidal elements included proteins, lipids, neutral polysaccharides, pectins and minerals. Colloidal elements amassed charge at their surface known as a surface potential. Surface potential was a key parameter affecting the strength of charge-based colloidal attraction of an element, most importantly electrostatic repulsion of other like charged elements (43). Elements accounted for the turbidity in a wine had an electrical charge. Core composition in bentonite was montmorillonite. Proteins in wine with isoelectric point values above the pH had a positive charge and freely absorbed with bentonite. Bentonite might indirectly combind phenols interacting with proteins and anthocyanins resulting to reduction of color (44). Bentonite was highly preferred by winemakers, as it was convenient in manipulation and application. It was very useful in removing yeast, tannins and other tough protein-based elements causing turbidity in wine (45). Polyvinylpolypyrrolidone was a high molecular weight of vinylpyrrolidone polymer. It could enhance the elimination of total polyphenols, and protein complexes. Mechanism of adsorption of polyvinylpolypyrrolidone interacting with polyphenols based on formation of hydrogen bonds between the phenolic groups and oxygen of the amine group of the pyrrolidone ring (46). Wheat gluten was composed of gliadins and glutenins (47). These biochemical attributes were accounted for protein-phenolic interactions, inducing to flocculation and clarification. Gluten provided better clarification than bentonite (48). Basic of gelatin was colloidal with a positive charge. It absorbed tannins with negative charge. In combination with the negatively charged elements, the incorporated mass acceleration induced deposit. Once this neutralization happened, the turbid elements propensity to flocculate making them to deposit. The efficacy of gelatin fining relied on polyphenols to elements proportion (43). Kaolin was clay effective in flocculation of yeast cells and abundant tannin inducing the wine more stable in warm preservation. Moreover, it was utilized to lighten colour and minimize harshness in the wine. Effectiveness of gluten wine clarification was similar to tannin-gelatin and more efficient than bentonite (48). Gelatin and kaolin were compared in clarifying fruit wines. Gelatin showed a better clarifier than kaolin (45). Bentonite and polyvinylpyrrolidone revealed significant impact on the elimination of turbidity in Merlot wine (46).

Table 6. Effect of clarifying agents (bentonite, polyvinylpyrrolidone, wheat gluten, gelatin, kaolin) at 0.03% (v/v) to turbidity (NTU), total phenolic content (mg GAE/100 ml), DPPH free radical scavenging (%) of wine

Parameters	Clarifying agent				
	Bentonite	Polyvinylpyrrolidone	Wheat gluten	Gelatin	Kaolin
Turbidity (NTU)	29.74±0.00^{ab}	22.06±0.01^{c}	31.15±0.02^{a}	24.41±0.00^{bc}	26.57±0.02^{b}
Ethanol (% v/v)	4.21±0.03^{a}	4.23±0.00^{a}	4.20±0.03^{a}	4.26±0.02^{a}	4.19±0.00^{a}
Total phenolic content (mg GAE/100 ml)	33.20±0.05^{a}	32.50±0.03^{b}	33.41±0.01^{a}	32.79±0.00^{ab}	33.01±0.01^{ab}
DPPH free radical scavenging (%)	12.27±0.01^{a}	11.31±0.00^{b}	12.38±0.03^{a}	11.84±0.01^{ab}	11.96±0.03^{ab}
Overall acceptance (sensory score)	8.64±0.01^{a}	7.92±0.00^{b}	8.75±0.03^{a}	8.34±0.01^{ab}	8.19±0.02^{ab}

Values are the mean of three replications; values in row followed by the same superscript lowercase letter/s are not differed significantly (α = P=0.05) by Duncan's multiple range test

Conclusion

We successfully utilized the ripen pond apple fruit to convert into wine as one value-added product. Technical varia-

bles such as sugar addition, yeast ratio, time and temperature in maceration, time in malolactic fermentation and fining agents in clarification were very important directly affecting to physicochemical attributes, phenolic content and antioxidant capacity of pond apple wine. Clarification was beneficial in separation of soluble dry matters like tannins, phenols and proteins. From clarification, fermentation process could be speeded up effectively to save production cost. High ethanol content and phytochemical retention contributed to the high sensory score of pond apple wine. These quality parameters were acceptable for an alcoholic drink. Success of this research also opened a new window for an improvement of income for people in rural area through exploitation of this underutilized fruit for processing factory as well as resolution of environmental polution.

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Compliance with ethical standards

Conflict of interest: The author strongly confirmed that this research is conducted with no conflict of interest.

Ethical issues: None.

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