Development of Fermented Prunus Vinegar: Chemical Characterization and Antioxidant Activity

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Abstract

The purpose of this study was to examine the chemical properties, antioxidant activities and sensory test of a two-stage fermented vinegar that was produced from three Prunus species, namely *Prunus persica* L., *Prunus domestica* L., and *Prunus mume L.* Alcoholic fermentation was produced using *Saccharomyces cerevisiae* and acetous fermentation was achieved using *Acetobacter pasteurianus*. Samples taken during the alcoholic fermentation showed a continuous decrease in total soluble solids and an increase in alcohol content at the end of fermentation process. The result showed that the wine produced from *Prunus persica* L. exhibited the highest content of alcohol (13.81±0.04 %, w/v) and exhibited the highest content of antioxidant activity (45.21±0.06 mg/ml). In the acetous fermentation, alcohol content dropped continuously and acetic acid content elevated at the end of the process. The highest content of acetic acid (4.04 ± 0.19 %, v/v) was detected in the vinegar produced from *Prunus domestica* L., while the vinegar produced from *Prunus persica* L. exhibited the highest content of antioxidant activity (49.08 ± 8.49 mg/ml). The 9-point hedonic scale showed that the vinegar produced from *Prunus mume L.* exhibited the highest overall acceptability (7.83±1.02), a result that indicated that consumers rated it at the very pleasant level on the preference scale.

Keywords: chemical properties; antioxidant activity; fruit vinegar; Prunus; sensory evaluation
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1. Introduction

Thailand is a tropical country that has an abundance of tropical fruits such as bananas, tamarinds and pineapples, which can be preserved to add value to fruit production by transforming fruit into functional foods, for example, banana vinegar [1]. In the north of Thailand, a lot of prunus fruit are grown and can be processed into healthy beverages. Prunus fruits species, include peach (*Prunus persica* L.), red plum (*Prunus domestica* L.), and chinese plum (*Prunus mume* L.) are mostly processed as frozen, caned, and dried fruits, as well as fruit juice. Currently, there is an upsurge of interest in new healthy foods, and this has stimulated the development of innovations in food processing, such as the production of novel vinegar drinks from fruits. Vinegar is rich in nutrients

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including amino acids, vitamins, sugars, organic acids, polyphenols, tetramethylpyrazine, and melanoidins [2]. The demand for fruit vinegars has increased because of its benefit as health food products, which offers various different kinds of beneficial effects to human such as having antibacterial, antidiabetic properties and lowering cholesterol levels by inhibiting the oxidation of low density lipoproteins (LDLs) [3].

Peach (*Prunus persica* L.) is a popular fruit appreciated by consumers for its odor and flavor and it is an excellent source of bioactive compounds such as phenolic compounds which show a variety portion of biological activities favorable to human health [4]. Red plum (*Prunus domestica* L.) is well-known worldwide because of its color, aroma, flavor and nutritional value. The main nutrition value of red plum comes from their phenolic compound such as phenolic acid and flavonoids, which decrease the risk of oxidative damage and cancer [5]. Chinese plum (*Prunus mume* L.) is a tree of genus Rosaceae. Chinese plum contains phenolic compounds, such as flavonoids and phenolic acids, which are involved in antioxidant activity. These phenolic compounds from Chinese plum also show an inhibitory effect against cancer cells [6].

Previously, bioactive compounds, volatile aroma compounds and antioxidant capacities of Sour cherry (*Prunus cerasus* L.) vinegars were studied [7]. However, there was not much information available in the literature concerning the production of vinegar from other prunus fruits. Therefore, the objective of the present work was to evaluate the chemical properties including alcohol contents, glucose and fructose contents, and acetic acid contents. Antioxidant activities were determined by DPPH radical assays, total phenolic contents and sensory scores of the Prunus vinegars was performed based on the 9-point hedonic scale test. Importantly, it was hoped that this research would enhance the utilization of the prunus fruits.

2. Materials and Methods

2.1 Chemicals and reagents

All the reagents and solvents used during these experiments were of analytical grade and were purchased from various suppliers, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was purchased from Sigma-Aldrich (Steinheim, Germany). Gallic acid standard was supplied by Fluka (Buchs, Switzerland). Folin-ciocalteau reagent was from Merck (Darmstadt, Germany) and sodium carbonate (anhydrous) from Univar (Downers Grove, IL, USA).

2.2 Raw materials

Peach (*Prunus persica* L.), Red plum (*Prunus domestica* L.) and Chinese plum (*Prunus mume* L.) fruits were harvested during March in 2019 at Chiang Mai province.

2.3 Prunus vinegar production

For the vinegar fermentation process, Prunus fruits of each species were pickled with rock sugar at a ratio of 1:1 w/w for 7 days. After that, the juice was squeezed from the fruits. The sugar content of the juice was then adjusted down to 24 ºBrix by addition of water. The Prunus juice was pasteurized for 30 min at 60ºC. Alcoholic fermentation was conducted for 5 days at room temperature in plastic vessels containing 2 l of the Prunus juice inoculated with wine yeast, *Saccharomyces cerevisiae* (Wine & Scientific Equipment Ltd., Part) at a ratio of 0.75% (v/v). The preparation of yeast inoculum was carried out by mixing 5 g of yeast powder with 60 ml of warm water. At the end of the fermentation process, the wine was separated from the sediment by allowing it to settle in glass bottles, followed by pasteurization for 30 min at 60 ºC and clarification for 45 days at 10 ºC. Then, the alcohol content of the obtained wine was adjusted to 7% (v/v) was inoculated with *Acetobacter pasteurianus* TISTR 102 (Thailand Institute of Scientific and Technological Research) which had been grown in glucose yeast extract broth at a ratio of 10% (v/v). The vinegar fermentation was carried out for 15 d at 30ºC on a shaker (150 g) in a glass flask containing 135 ml of the Prunus wine. The samples were allowed to settle in microtubes and storage at 4 ºC before the analyses.

2.4 Chemical analysis

The wine and vinegar samples were centrifuge and filtered through a 0.45 µm filter before injection into HPLC system. The analysis were performed on a Shimadzu HPLC-RID system (Shimadzu, Japan) consisting of Shimadzu LC-20AD pumps and RID-10A refractive index detector. The analytical columns used were Aminex HPX-87H column (300 mm × 7.8 mm i.d., 9 µm, Bio-Raand Laboratories, Inc., USA) coupled to a cationic exchange precolumn (Bio-Rad Laboratories, Inc., USA). H_2SO_4 (5 mM) was used as the mobile phase. The injection volume was 20 ml with a flow rate of 0.6 ml/min. The column temperature was set at 45ºC. [8]. A series of standard solutions (ranging from 0-12 % w/v of fructose, glucose and 0-16 % v/v alcohol, acetic acid) were prepared. A standard curve with R^2 greater than 0.99 was plotted, and then the concentrations of sugar, alcohol and acetic acid in wine and vinegar were quantified accordingly. The total soluble solids values of the wine were measured using refractometer (AllA France, France) calibrated with distilled water. The values were expressed as ^{O}Brix.

2.5 Antioxidant activity

The antioxidant activities of the sample were determined by DPPH radical assay [9] in which 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical was used as a stable radical. In brief, 5 ml of each sample was added to 5 m of 0.1 mM DPPH radical solution prepared in ethanol, and the mixture was incubated for 20 min at room temperature in the dark. After incubation, absorbance was measured at 517 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Japan), and the DPPH radical scavenging activities were expressed as mg ascorbic acid equivalents in 1 ml of sample (mg/ml).

2.6 Total phenolic content analysis

Folin-Ciocalteu method was utilized for the determination of total phenolic contents of the Prunus vinegars [10]. Briefly, 1 ml of each sample was diluted with 9.5 ml of distilled water and was then mixed with 0.5 ml of Folin-Ciocalteu reagent and 2 ml of 10% Na_2CO_3 solution. After 30-min incubation at room temperature, absorbance was measured at 765 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Japan). Results were expressed as mg gallic acid equivalents in 1 l of sample (mg GAE/l).

2.7 Sensory evaluation

Drinking vinegar was prepared by 200 g of the Prunus vinegars, 150 g of honey and 150 g of water were mixed together to make drinking vinegars and the drinking vinegars were subjected to the sensory evaluation based on the 9-point hedonic scale by using 30 untrained panelists for 5 attributes of clarity, color, odor, taste and overall acceptance. A scale value of 9 represented like extremely, 5 represented neither like nor dislike and 1 represented dislike extremely.

2.8 Statistical analysis

The trials were carried out in triplicate. The results were given as the mean ± standard deviation (SD). The obtained data were analyzed by one-way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) to determine the significance between samples. In all cases, $p < 0.05$ was considered significant.

3. Results and Discussion

3.1 Chemical properties of the Prunus wines and vinegars

The Prunus wines produced from three Prunus species via a 5-day alcoholic fermentation process using *Saccharomyces cerevisiae* as an inoculant were analyzed for their chemical compositions, and the results are presented in Tables 1-8. It was observed that at the end of the fermentation, high alcohol content was detected in peach wines, indicating that sugars in the peach juice had been rapidly converted to alcohol. The Prunus wine produced from 'peach' species contained the highest alcohol content of 13.81 ± 0.04 %, v/v which was similar to that (8.12 %) detected in peach wines produced from Redhaven variety [11]. As given in Table 2, glucose was rapidly utilized during the production of the Prunus wine as observed for peach. Notably, glucose was completely depleted in peach wine samples after 5 days of the fermentation. Fructose was likely to be utilized more slowly as compared to glucose (Table 3). The most rapid utilization of fructose was observed in the Prunus wine produced from peach, which was depleted in day 5 of the fermentation. Yeasts fermented only hexose sugars. Yeasts fermented sugars at quantity up to 20%; at higher concentrations the metabolism slow down. During alcoholic fermentation, sugars must be metabolized to pyruvate via the glycolytic pathway, which is then decarboxylated to acetaldehyde and finally reduced to ethanol. Glucose and fructose were the favored sugars of *S. cerevisiae* [12]. In Table 4, the total soluble solids of 3 wine were adjusted to 24 ºBrix, and after fermentation for 5 days, the results showed that peach wine (7.30 ºBrix) had less TSS than Chiness plum (14.50 ºBrix) and Red plum (13.50 ºBrix). From the experiment, it was found that the alcohol content in Peach wine was higher than Chinese Plum and Red plum wine because yeast had the ability to consume TSS in peach effectively. During a 15-day acetous fermentation process, oxidative fermentation is a fermentation process caused by bacteria that require oxygen for respiration at the cellular level. Acetic acid bacteria, for examples *Acetobacter pasteurianus*, were used as the starter cultures for producing vinegar. In acetous fermentation, the mechanism for the conversion of alcohol into acetic acid by alcohol dehydrogenase has pyroloquinoline quinone (PQQ) and acetaldehyde dehydrogenase (ALDH) as cofactors. Under these conditions, acetic acid will be excreted and accumulated in media culture until ethanol was completely oxidized [13]. In our work, the Prunus vinegars produced from the three Prunus wines using *A. pasteurianus* were analyzed for their chemical compositions, and the results are given in Tables 5-8. All the Prunus vinegars showed a significant decrease in the alcohol content as it was converted to acetic acid by acetic acid bacteria. However, the alcohols were not completely depleted, in which at the end of acetous fermentation the vinegar produced from peach contained the highest alcohol content of 3.35%, v/v while that produced from red plum had the lowest alcohol content of 1.14%, v/v. For the acetous fermentation, at the end of a 15-day acetous fermentation process, acetic acid content was found to range from 2.90% to 4.04%, v/v with the highest value of 4.04%, v/v, observed in the Prunus vinegar produced from Red Plum, a result which was much higher those found in mulberry vinegar (3.90%) [14] and banana vinegar (3.49%) [1].

Table 1. Changes in alcohol contents of the Prunus wines produced via alcoholic fermentation process

Species	Alcohol content (%, v/v)					
	Days after fermentation					
	0	1	2	3	4	5
Chinese plum	0	3.02 ± 0.24[b]	3.94 ± 0.04[c]	4.71 ± 0.01[c]	6.20 ± 0.05[c]	6.23 ± 0.01[c]
Red plum	0	4.38 ± 0.03[a]	5.59 ± 0.00[b]	5.35 ± 0.00[b]	6.71 ± 0.01[b]	6.72 ± 0.01[b]
Peach	0	2.53 ± 0.05[c]	7.78 ± 0.02[a]	10.29 ± 0.05[a]	12.34 ± 0.23[a]	13.81 ± 0.04[a]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 2. Changes in glucose contents of the Prunus wines produced via alcoholic fermentation process

Species	Glucose content (%, w/v)					
	Days after fermentation					
	0	1	2	3	4	5
Chinese plum	17.67 ± 0.01[a]	15.83 ± 0.12[a]	10.5± 0.00[a]	8.74 ± 0.00[a]	6.83 ± 0.01[a]	6.83 ± 0.01[a]
Red plum	11.82 ± 0.01[b]	9.92 ± 0.01[b]	8.79 ± 0.01[b]	7.79 ± 0.01[b]	6.42 ± 0.01[a]	5.79 ± 0.01[b]
Peach	10.16 ± 0.02[c]	8.91 ± 0.03[c]	2.92 ± 0.01[c]	1.83 ± 0.01[c]	0.86 ± 0.29[b]	0.00[c]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 3. Changes in fructose contents of the four berry wines produced via alcoholic fermentation process

Species	Fructose content (%, w/v)					
	Days after fermentation					
	0	1	2	3	4	5
Chinese plum	21.00 ± 0.07[a]	19.00 ± 0.01[a]	16.43 ± 0.46[a]	14.13 ± 0.09[a]	12.7 ± 0.00[b]	12.73 ± 0.02[a]
Red plum	16.58 ± 0.48[b]	14.74 ± 0.01[b]	14.78 ± 0.65[b]	14.29 ± 0.47[a]	13.92 ± 0.15[a]	12.17 ± 0.12[b]
Peach	15.77 ± 0.03[c]	14.11 ± 0.43[b]	7.46 ± 0.01[c]	4.50 ± 0.00[b]	0.48 ± 0.36[c]	0.00[c]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 4. Changes in total soluble solid of the Prunus wines produced via alcoholic fermentation process

Species	**Total soluble solid (ºBrix)**					
	Days after fermentation					
	0	1	2	3	4	5
Chinese plum	24	23.00 ± 0.00^{a}	20.50 ± 0.71^{a}	18.80 ± 0.28^{a}	17.00 ± 0.00^{a}	14.50 ± 0.71^{a}
Red plum	24	21.50 ± 0.71^{b}	18.70 ± 0.14^{a}	16.50 ± 0.71^{b}	15.00 ± 0.00^{b}	13.50 ± 0.71^{a}
Peach	24	20.00 ± 0.00^{c}	13.50 ± 0.71^{b}	8.50 ± 0.71^{c}	8.00 ± 0.00^{c}	7.30 ± 0.14^{b}

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 5. Changes in acetic acid contents of the Prunus vinegars produced via acetous fermentation

Species	**Acetic acid content (%, v/v)**			
	Days after fermentation			
	0	5	10	15
Chinese plum	0.24 ± 0.12	1.43 ± 0.12^{a}	2.06 ± 0.14^{a}	3.88 ± 0.70
Red plum	0.10 ± 0.00	1.03 ± 0.00^{b}	3.41 ± 0.18^{a}	4.04 ± 0.19
Peach	0.09 ± 0.00	0.20 ± 0.01^{c}	0.88 ± 0.68^{b}	2.90 ± 0.06

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 6. Changes in alcohol contents of the Prunus vinegars produced via acetous fermentation

Species	**Alcohol content (%, v/v)**			
	Days after fermentation			
	0	5	10	15
Chinese plum	5.80 ± 0.00^{b}	3.70 ± 0.02^{b}	2.84 ± 0.12^{b}	1.61 ± 0.00^{b}
Red plum	6.49 ± 0.34^{b}	4.95 ± 0.03^{c}	2.40 ± 0.00^{c}	1.14 ± 0.02^{c}
Peach	8.14 ± 0.00^{a}	7.19 ± 0.03^{a}	5.34 ± 0.00^{a}	3.35 ± 0.01^{a}

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 7. Changes in glucose contents of the Prunus vinegars produced via acetous fermentation

Species	**Glucose content (%, w/v)**			
	Days after fermentation			
	0	5	10	15
Chinese plum	2.77 ± 0.0.1^{a}	2.96 ± 0.00^{a}	2.99 ± 0.01^{a}	3.14 ± 0.01^{a}
Red plum	2.45 ± 0.00^{b}	2.46 ± 0.00^{b}	2.41 ± 0.01^{b}	2.41 ± 0.00^{b}
Peach	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 8. Changes in fructose contents of the Prunus vinegars produced via acetous fermentation

Species	**Fructose content (%, w/v)**			
	Days after fermentation			
	0	5	10	15
Chinese plum	5.80 ± 0.08[a]	6.08 ± 0.07[a]	6.06 ± 0.07[a]	6.73 ± 0.15[a]
Red plum	5.59 ± 0.02[a]	5.77 ± 0.02[b]	5.76 ± 0.07[b]	5.90 ± 0.09[b]
Peach	0.00 ± 0.00[b]	0.00 ± 0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

3.2 Total phenolic contents and antioxidant activities

The antioxidant activity of fruit is attributed to the presence of phytochemical compounds such as ascorbic acid and carotenoids. The polyphenols are the primary antioxidant compounds of various fruits [15]. Two roles of antioxidants are to inhibit lipid oxidation and to scavenge free radicals, and methods used to determine the antioxidant activity in wine and vinegar are based on the free radical scavenging activity with DPPH. The levels of antioxidant activities of the Prunus vinegars are presented in Table 9. The results showed that the Prunus wine derived from peach exhibited the highest antioxidant activity of 75.21 mg/ml which was less than that produced from Redhaven cultivar peach (387.95 mg/ml) [11]. Similarly, the vinegar produced from peach species was observed to exhibit the highest antioxidant activity of 49.08±8.49 mg/ml which was much higher than that detected in Rich Lady Peach (3.50 g/kg) [16]. The levels of total phenolic contents detected in the Prunus vinegars produced from different Prunus species via the two-stage fermentation process are given in Table 10. It was noted that the Prunus wine derived from Red plum contained the highest levels (215.85±1.43 mg/l) of total phenolics. Similar results were observed for the Prunus wine produced from the same species, in which the vinegar measured at the end of acetous fermentation exhibited the highest total phenolic content of 133.08±0.76 mg/l, which was much less than that detected in prune juice (441±59 mg/l), in which the main polyphenol in prunes juice were hydroxycinnamates, neochlorogenic acid and chlorogenic acid [17]. The antioxidant activity and total phenolic content were decreased in vinegar, and this result was in agreement with an earlier study of Towantakavanit *et al*. [18] that demonstrated that the decrease in total phenol level could have been due to fermentation process condensation and polymerization reactions as well as the formation of oxidative products and precipitations.

Table 9. Antioxidant activities of the three Prunus wine and vinegars produced via a two-stage fermentation process

Species	**DPPH (mg/ml)**	
	Wine	Vinegar
Chinese Plum	5.42 ± 0.00[c]	25.83 ± 0.00[b]
Red Plum	14.96 ± 0.18[b]	27.67 ± 0.82[b]
Peach	45.21 ± 0.06[a]	49.08 ± 8.49[a]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 10. Total phenolic contents of the three Prunus wine and vinegars produced via a two-stage fermentation process

Species	Total phenolic content (mg/l)	
	Wine	Vinegar
Chinese Plum	66.39 ± 0.67[c]	36.66 ± 0.29[c]
Red Plum	215.85 ± 1.43[a]	133.08 ± 0.76[a]
Peach	140.51 ± 0.38[b]	80.78 ± 0.57[b]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

3.3 Sensory evaluation

The levels of consumers' preference based on the 9-point hedonic scale of the vinegar drinks, a blend of the vinegars made from different Prunus species and honey, are depicted in Table 11. The results show that significant ($p < 0.05$) differences in clarity, color, odor taste and overall acceptability were observed among the drinking vinegars produced from different Prunus species. The drinking vinegar produced from Chinese plum displayed the highest level of consumers' preference, with the mean overall acceptability score of 7.83 ± 1.02, which was equivalent to the hedonic scale of 9. In our study, the high levels of consumers' preference were for the Chinese plum vinegar, and this might be because the taste of the vinegar was sweet blended with sour and because the flavor included some alcohol odor.

Table 11. Sensory scores of the drinking vinegars blended from the three fermented Prunus vinegars

Species	Clarity	Color	Odor	Taste	Overall acceptability
Chinese Plum	6.70 ± 1.21 [ns]	6.77 ± 1.33[ns]	7.20 ± 1.37 [ns]	7.27 ± 1.41 [ns]	7.83 ± 1.02[a]
Red Plum	6.80 ± 1.42 [ns]	6.50 ± 1.25[ns]	6.67 ± 1.60 [ns]	6.70 ± 1.29 [ns]	7.03 ± 1.30[b]
Peach	6.40 ± 1.52 [ns]	6.53 ± 1.83[ns]	6.57 ± 2.03 [ns]	6.77 ± 1.98 [ns]	6.57 ± 1.79[b]

Values with various letters in the same column are significantly different according to Duncan's multiple range test ($p < 0.05$).

4. Conclusions

This study was conducted in order to compare the levels of acetic acid, total phenolics, antioxidants and consumers' preference of the Prunus vinegars produced from three Prunus species via a two-stage fermentation process. The results showed that the vinegars produced from Red plum species exhibited the highest level of acetic acid (4.04 %, v/v), while those produced from peach displayed the highest antioxidant activities (49.08 mg/ml), as measured by means of DPPH radical assay. Meanwhile, the vinegars produced from Red plum were observed to have the highest total phenolics

(133.08 mg/l). Sensory test based on the 9-point hedonic scale using untrained panelists showed that the drinking vinegars made from Chinese plum had the highest overall preference (7.83).

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