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Comparative chemical properties and antioxidant activity of two types of pomegranate fermented vinegar

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Abstract

This study was conducted to produce fermented vinegar from two types of pomegranate, namely Chinese and Indian cultivar. The aim of the present study was to examine the chemical properties of vinegar and its antioxidant activities. From the fermentation of wine for 9 days, Chinese pomegranate wine had 9.95 ± 0.28 percent of alcohol content. The highest antioxidant activities and total phenolic content observed for the wine produced from the Indian cultivar were 32.58 ± 0.68 mg/mL and 536.73 mg/L, respectively. Vinegar fermentation was operated by inoculating 10% (v/v) of *Acetobacter pasteurianus* into two pomegranate wines with their start alcohol content adjusted to 4% (v/v). The fermentation was proceeded for 15 days at 30 °C and sampling was operated at 5-day intervals. It was remarked for all samples that the level of alcohol decreased continuously over the fermentation period, While the amount of acetic acid increased. At the end of the fermentation process, the fermented vinegar produced from the Chinese cultivar showed the highest level of acetic acid of 7.50 ± 0.21%. The highest antioxidant activities and total phenolic content observed for the vinegar produced from the Indian cultivar were 35.25 ± 0.90 mg/mL and 480.24 ± 28.82 mg/L, respectively. The 9-point hedonic scale showed that the vinegar produced from Indian pomegranate exhibited the highest overall acceptability (7.63 ± 1.24), which indicated the very much pleasant level of the vinegar preference of the consumers.

Keywords: Wine, Fermented vinegar, Pomegranate, Antioxidant activity

1. Introduction

The scientific name of pomegranates is *Punica granatum* L. family Punicaceae. Pomegranate is native to eastern Iran, in southern Afghanistan. Pomegranate fruit is parallel a "superfood" because it contains many phytochemicals such as antioxidants, polyphenols, punicalins, punicalagins, anthocyanis, ascorbic acid, citric acid, fumaric acid, malic acid, essential amino acids, vitamins B and C [1]. A pomegranate is a fruit that is rich in antioxidants like ellagitannins, flavonoids, and polyphenols [2]. Pomegranate synthesizes antioxidants compounds to prevent the danger of pollution in the environment. Pomegranate juice can reduce the content of low-density lipoproteins (LDLs) that can cause arteriosclerosis and lead to heart disease [3] and prevent oxidative stress such as cancer [4]. Vinegar is rich in nutrients and bioactive compounds, including amino acids, sugars, organic acids, polyphenols, melanoidins, and tetramethylpyrazine [5]. Vinegar is a highly beneficial drink to promote different kinds of beneficial effects to consumers, such as having antidiabetic effects and lowering cholesterol levels in blood by inhibiting the oxidation of LDL [6]. Fruit vinegar is a vinegar obtained from the fermentation of various fruits. The production of vinegar has two fermentation steps: the alcohol fermentation by using yeast convert sugar to alcohol in anaerobic conditions followed by acetous fermentation to form acetic acid with *Acetobacter* and *Gluconobacter* in the aerobic condition and the acetic acid content of vinegar is not less than 4%, In the cell, acetic acid was absorbed from the intestine and metabolized to acetyl CoA in the liver, fructified in the feed-forward activation of Adenosine Monophosphate (AMP)-activated

protein kinase (AMPK). AMPK was a member of the metabolite-sensing protein kinase family and has been related to the regulation of enzyme activities involved in energy metabolism thus, AMPK has been recognized as a promising target for the management of obesity [7,8]. Nowadays, fermented vinegar is a popular beverage as a drink with honey and warm water to help the digestive system better. Pomegranate can be processed into a healthy drink, and it increases the value of pomegranates. This study aimed to compare the chemical properties and antioxidant activity of two pomegranate vinegar. The results of this study can contribute to raw material for the development of new pomegranate products.

2. Materials and methods

2.1 Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was purchased from Sigma–Aldrich (Steinheim, Germany). Folin-ciocalteau reagent was from Merck (Darmstadt, Germany) and sodium carbonate (anhydrous) from Univar (Downers Grove, IL, USA). Deionized water was prepared by a Milli-Q Water Purification System (Millipore, MA, USA).

2.2 The production of pomegranate vinegar

Pomegranate seeds of each cultivar were crushed and mixed with water at a ratio of 1:1 to prepare pomegranate juice. After adjustment of total soluble solid content up to 20 ºBrix by cane sugar, the pomegranate juice was pasteurized for 30 min at 60 °C. Alcoholic fermentation was conducted for 9 days at room temperature under static conditions in plastic vessels containing 3 L of the pomegranate juice inoculated with Red star (premier classique) wine yeast, *Saccharomyces cerevisiae*, (Wine & Scientific Equipment Ltd., Part., Ratchaburi, Thailand) at a ratio of 0.75% (v/v). Preparation of yeast inoculum was carried out by mixing 5 g of yeast powder with 80 mL of warm water. At the end of the fermentation process, the obtained wine was separated from the sediment by allowing it to settle in glass bottles, followed by pasteurization for 30 min at 60 °C and clarification for 45 days at 10 °C. Prior to acetous fermentation, the alcohol content of the obtained wine was adjusted to 4% by sterile distilled water. Acetous fermentation was performed for 15 days under the aforementioned conditions in glass vessels containing 135 mL of the pomegranate wine inoculated with 10% (v/v) *Acetobacter pasteurianus* TISTR 521 (Thailand institute scientific and technological research) which was grown in glucose yeast broth (GYB) for 1 day at 30 °C on an shaker (150 rpm) in glass flask. The fermentation was carried out for 15 days at 30 °C on an shaker (150 rpm) in glass flask containing 135 mL of the pomegranate wine. Sampling was settled in a microtube and stored at 4 °C before the analyses.

2.3 Chemical analysis

Analysis of alcohol, acetic acid, glucose, and fructose contents was performed on a Shimadzu HPLC-RID system (Shimadzu, Japan) consisting of Shimadzu LC-20AD pumps RID-10A refractive index detector. The analytical column was Aminex HPX-87H column (300 mm × 7.8 mm i.d., 9 µm, Bio-Rad 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 0.2 mL with a flow rate of 0.6 mL/min. The column temperature was set at 45 °C. A series of a standard solution (ranging from 0-16 % v/v of fructose, glucose, ethanol and acetic acid) were prepared. A standard curve with R^2 greater than 0.99 was plotted, and then the concentration of the sugar, alcohol and acetic acid in wine and vinegar were quantified accordingly [9].

2.4 Total phenolic contents

Total phenolic contents of the pomegranate vinegar were determined using Folin-Ciocalteu reagent [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 mL of sample (mg GAE/L).

2.5 DPPH radical-scavenging activity

The vinegar's antioxidant activities were evaluated by 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical assay [11] in which DPPH radical was used as a stable radical. In brief, 1.5 mL of each sample was added to 1.5 mL 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 in1 mL of sample (mg/mL).

2.6 Sensory analysis

About 200 g of the pomegranate vinegar were mixed with 150 g of honey and 150 g of water to make the vinegar drink, and the obtained vinegar was subjected to the sensory evaluation based on the 9-point hedonic scale by using 30 untrained panelists. The panelists were asked to rank the 9-point scale of affective tests of clearance, color, odor, taste, and overall acceptance with the scale 9 representing extremely like, 5 representing neither like nor dislike, and 1 representing dislike extremely. [12]

2.7 Statistical analysis

A randomized block design, with three replicates and two samples per replicate, was used to compare the chemical properties, antioxidant activities, and consumers' preference of the pomegranate vinegar produced from two pomegranate cultivars. The results are expressed as the mean ± one standard deviation (SD) of three replicates, and data were analyzed using an independent t-test to determine the significance between samples. In all cases, $p < 0.05$ was considered significant.

3. Result and discussion

2.1 The chemical quality of the wine and vinegar during the fermentation

3.1.1 Alcohol fermentation

According to chemical analysis results of alcohol content, glucose content, and fructose content, it was found that on the 9th, the wine produced from the Chinese pomegranates had the highest alcohol content (9.95% ± 0.28%) (Figure 1). The alcohol content of wine was due to the metabolism of yeast to convert sugars to ethanol [13]. The results showed that pomegranate wine had alcohol content higher than the Mollar de Elche pomegranate (9.10%) where the fermentation takes 6 days [2]. On the 5th of fermentation, glucose content was zero in the fermentation of Chinese pomegranate wines. Moreover, the yeast was able to use sucrose efficiently in the Chinese cultivar of pomegranates. According to the alcohol fermentation to produce alcohol by converting pyruvate into ethanol in the absence of oxygen gas through the process of glycolysis (Glycolysis pathway), the yeast used the sugar to grow in order to increase cell content, and some were used to synthesize other metabolites such as ethanol, carbon dioxide, acetic acid, lactic acid, and succinic acid. [6]

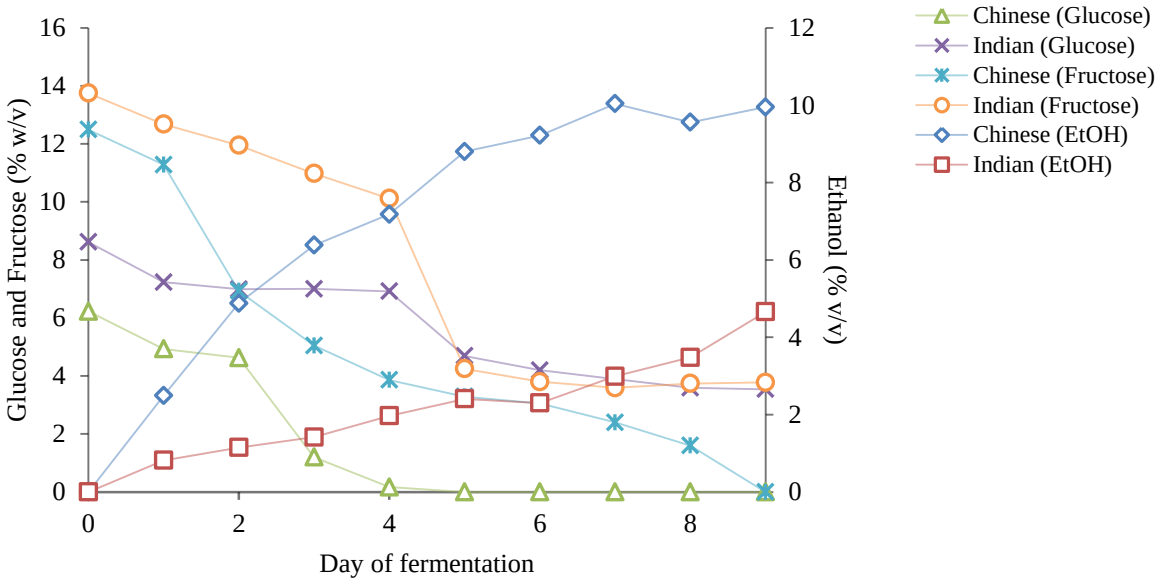

Figure 1 Physicochemical properties of pomegranate wine from 2 cultivars during a 9-day fermentation process.

3.1.2 Acetous fermentation

According to the alcohol content and acetic acid content from vinegar production from both cultivars of Chinese and Indian pomegranates, the fermentation process took 15 days. It was found that on the 15th day of Chinese pomegranate vinegar fermentation, the remain of alcohol content was 0.85% ± 0.04% (Figure 2). The acetic acid content was found at day 15 of Chinese pomegranate vinegar fermentation was 7.50 ± 0.21%. Chinese vinegar contained more acetic acid than pomegranate vinegar (3.38 ± 0.03%) [14]. Pomegranate vinegar (4.90%) which the final alcohol concentration was targeted to be 8-12% (v/v) [15].

The fermentative oxidation of ethanol to acetic acid had been presented to depend on two sequential reactions of membrane-bound pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) and aldehyde dehydrogenase (ALDH). PQQ-ADH was a member of the alcohol dehydrogenase family and was essential for AAB oxidation of ethanol into acetic acid. The metabolism of acetic acid in *A. pasteurianus* included the Embden-Meyerhof-Parnas (EMP) pathway, pentose phosphate pathway (PPP), pyruvate metabolism pathway, ethanol oxidation respiratory chain pathway, and tricarboxylic acid cycle pathway (TCA) [16]. The glucose and fructose content showed in Figure 2, results revealed that the glucose content of Indian pomegranate vinegar throughout the fermentation period was not remarkably different ($p > 0.05$).

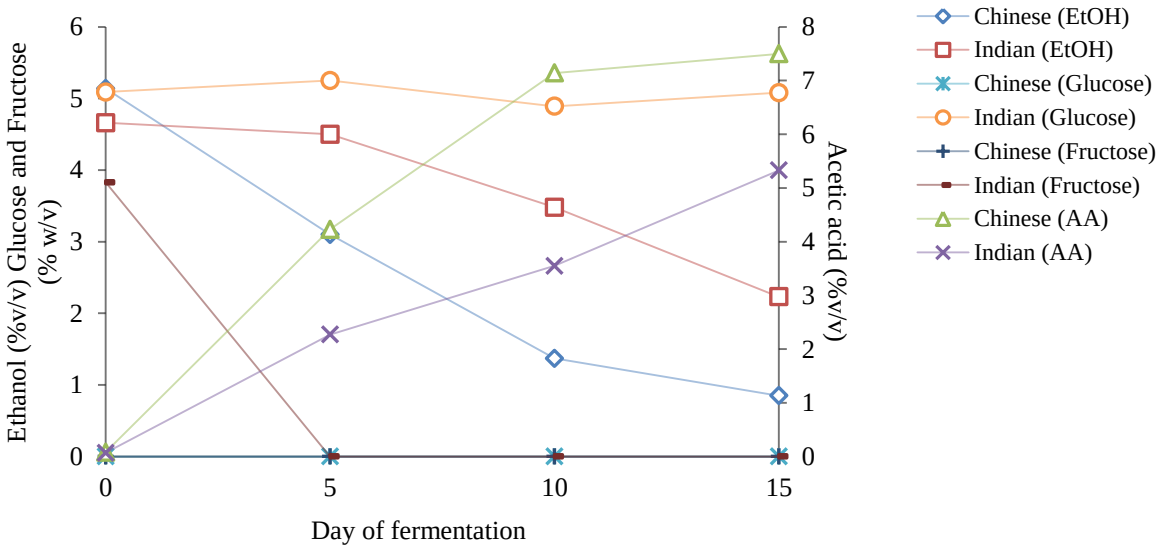

Figure 2 Physicochemical properties of Pomegranate vinegar from 2 cultivars during a 15-day fermentation process.

3.2 Antioxidant activity of wine and two cultivars of pomegranate vinegar

The DPPH method is a single-electron-transfer reaction which measures the antioxidant reducing capacity. It is simple and inexpensive. The DPPH method was tested for the antioxidant activity of wine and vinegar made from 2 cultivars of pomegranate vinegar. The antioxidant activity played a role in preventing diseases such as cancer, heart disease, fat embolism, cataract, and the antioxidants are also ingredients of health supplements. The research shows that wines made from Chinese and Indian cultivar of pomegranates had the antioxidant activity of 20.94 ± 1.85 and 32.58 ± 0.68 mg/mL, respectively. The wine produced from Indian pomegranates had more antioxidant activity than Wonderful pomegranate wine (4.38 mg/mL) [2], and the antioxidant activity of Indian pomegranate vinegar was 35.25 ± 0.90 mg/mL (Table 1). It was found that the antioxidant activity of Indian pomegranate vinegar was greater than pomegranate vinegar (1.54 mg/mL) [15] and Chinese pomegranate vinegar.

Table 1 Antioxidant activity of the two cultivars of pomegranate vinegar produced via a two-stage fermentation process.

Cultivars	DPPH (mg/mL)	
	Wine	Vinegar
Chinese pomegranate	20.94±1.85^{b}	28.67±2.15^{b}
Indian pomegranate	32.58±0.68^{a}	35.25±0.90^{a}

^{a,b} Values with various alphabets in the duplicate column are remarkably different ($p < 0.05$).

3.3 Total phenolic content of wine and vinegar from two cultivars of pomegranates

For determining the total phenolic content of pomegranates, the total phenolic content of wine made from Chinese and Indian cultivar pomegranate was 286.64 ± 1.71 mg/L and 536.73 mg/L, respectively. It was found that wine made from Indian pomegranates had a higher total phenolic content than cranberry wine (518.26 ± 11.25 mg/L) [17], and the total phenolic content of Indian pomegranate vinegar was 480.24 ± 28.82 mg /L (Table 2). According to [18], it was informed that there were 16 phenolic compounds in pomegranate vinegar, including Gallic acid, galloylglucoside, protocatechuic acid, punicalagin, catechin, vanillic acid, syringic acid, ethyl gallate, ellagic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, ferulic acid hexoside, tyrosol and trans-p-Coumaric derivate measured by ultra-performance liquid chromatography-mass spectrum (UPLC-MS). In the present study, the antioxidant activity and total phenolic content of Indian pomegranate wine and vinegar were higher than Chinese pomegranate wine and vinegar. Previous study by [19] exhibited the total phenolic content of Indian-Mridula pomegranate aril was 26.38 ± 3.50 mg GAE/g and previous study by [20] exhibited the total phenolic content of Chinese pomegranate aril was 0.39 ± 0.01 mg GAE/g.

Table 2 Total phenolic content of the two cultivars of pomegranate vinegar produced via a two-stage fermentation process.

Cultivars	Total phenolic content (mg/L)	
	Wine	Vinegar
Chinese pomegranate	286.64±1.71[b]	144.12±17.90[b]
Indian pomegranate	536.73±0.00[a]	480.24±28.82[a]

[a,b] Values with various alphabets in the duplicate column are remarkably different ($p < 0.05$).

3.4 The sensory test of pomegranate vinegar drink

The levels of consumers' preference based on the 9-point hedonic scale of the vinegar drinks blended of the vinegar made from pomegranate vinegar, water, and honey displays in Table 3. The results showed that significant ($p < 0.05$) differences in sweet and overall acceptability were observed among the vinegar drink produced from two types of pomegranate cultivars. The vinegar drink produced from Indian pomegranates displayed the level of consumers' preference, with the mean overall acceptability score of 7.63 ± 1.24. In our study, the high levels of consumers' preference of Indian pomegranate vinegar drink might be because the taste of the vinegar was sweet blended with sour, and the color was a plum purple color.

Table 3 Sensory scores of the vinegar drink blended from the two cultivars of pomegranate vinegar.

Cultivars	Color	Odor	Sweet	Sour	Overall acceptability
Chinese pomegranate	7.13±1.52	6.67±1.47	6.53±2.14	6.43±1.87[b]	6.87±1.55[b]
Indian pomegranate	6.50±1.50	6.40±2.01	7.60±1.25	6.10±1.84[a]	7.63±1.24[a]

[a,b] Values with various alphabets in the duplicate column are remarkably different ($p < 0.05$).

4. Conclusion

This study compared chemical properties, antioxidant activity, and sensory tests of two fermented pomegranate vinegar. It was found that the vinegar made from Chinese pomegranates had the highest acetic acid content (7.50% ± 0.21%). The antioxidant activity showed that the Indian pomegranate vinegar had the highest antioxidant activity (35.25 ± 0.90 mg/mL) and total phenolic content, respectively (480.24 ± 28.82 mg/L). The sensory test based on the 9-point hedonic scale using untrained panelists showed that the vinegar drink made from Indian pomegranates had the highest overall preference (7.63). Pomegranate vinegar can be a good source of nutrients which can be used to treat various diseases and it can be develop to the new nutritional supplements.

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