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Research article

Physicochemical characteristics of an alcohol hangover relief drink containing persimmon vinegar

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Abstract: The development of alcohol hangover relief drinks by adding persimmon vinegar was investigated in this study. This study aimed to develop and investigate the physicochemical characteristics of a hangover relief drink, derived from persimmon vinegar, that may have health benefits. Persimmon vinegar was added at concentrations of 0, 2.5, 5, 7.5, 10 and 12.5%. The higher the concentration of persimmon vinegar, the lower the pH, °Brix and reducing sugar content of the product. In contrast, higher titratable acidity, turbidity and tannin values were obtained with increasing concentrations of persimmon vinegar. The addition of 12.5% persimmon vinegar induced the highest alcohol dehydrogenase and acetaldehyde dehydrogenase activities at 160.91 and 117.14%, respectively. The L value also decreased as persimmon vinegar concentration increased. The addition of persimmon vinegar at high concentrations decreased fructose, glucose and maltose content but increased the sucrose content of the drink. Ca, K and Na were the most abundant minerals in the drink. Some organic acids, such as oxalic, malic, lactic, acetic, citric and succinic acids, were also detected in the developed alcohol hangover relief drink. This study suggests that adding 7.5% of persimmon vinegar improves the physicochemical characteristics, especially the Alcohol dehydrogenase and aldehyde dehydrogenase activities. This finding indicates that this formulated drink with 75% persimmon vinegar may be beneficial against oxidative stress.

Keywords: beverage; extraction; persimmon; vinegar; alcohol hangover relief drink

1. Introduction

Vinegar can easily be found in the market. Commercially, the two types of vinegar are cider and regular vinegar. Cider vinegar is made from fruit juices [1], whereas regular vinegar is made from raw plant materials, such as grains, apples, grapes or sugar cane [2]. Alcoholic fermentation (in the presence of yeast, such as *Saccharomyces cerevisiae*) and acetous fermentation (in the presence of acetic acid bacteria) are the two main biotechnological processes in vinegar production [3]. Alcoholic fermentation converts sugar to alcohol, whereas acetous fermentation converts it to acetic acid. The FDA recommends that natural vinegars should contain over 4 g of acetic acid per 100 mL. The nutritional constituents of vinegar include amino acids, sugars, vitamins and microelements [4]. Among the domestic standards of vinegar, persimmon vinegar contains > 2.6% acetic acid compared to other vinegars that contain 2.0–4.0% acetic acid. The U.S. Food and Drug Administration standards require vinegar to contain at least 4% acetic acid. Persimmon vinegar is known to have various effects, including those related to fatigue recovery by activation of metabolic functions in the body, prevention of osteoporosis and obesity, and elimination of hangovers by activation of alcohol decomposition.

Chronic alcohol consumption can cause liver cell disorders that affect metabolism and have adverse effects on primary organs, as well as the gastrointestinal, nervous, endocrine, hematopoietic and immune systems. Alcoholism can also cause fatty liver disease, which results in alcoholic hepatitis and cirrhosis. However, the pathogenesis of ethanol-based liver diseases remains unclear. In addition to the toxic effects of alcohol, various factors contribute to its pathogenesis, such as direct or indirect nutritional disorders, genetics and immunological mechanisms.

Several drugs have been developed to reduce or eliminate hangovers after drinking, in response to recent social trends in drinking culture. However, their toxicity and side effects necessitate the development of healthy beverages containing safe natural products. In Korea, persimmon vinegar is prepared using *Acetobacter* sp. It has been reported that persimmon vinegar with a titratable acidity content of 7% can be produced by acetic acid fermentation for 10–12 days after alcohol fermentation of persimmon pulp for five days, as a two-step process of alcohol and acetic acid fermentation [5]. This study aimed to develop a hangover relief drink with health benefits by producing vinegar through a traditional fermentation method, selecting herbal medicines that aid in fatigue recovery, establishing optimal extraction conditions, adding vinegar to the extract and investigating the chemical characteristics of the drink.

2. Materials and methods

2.1. Preparation of alcohol hangover relief drink with persimmon vinegar addition

The ingredients used in this experiment, such as *Hovenia dulcis*, *Alnus japonica*, *Sorbus commixta*, *Schisandra chinensis*, Pueraria Root, persimmon vinegar and jujube, were purchased from Jecheon Pharmaceutical Micro Farming Corporation, Korea. The mixture was prepared by combining all ingredients with tap water (30 times the total weight of the plant materials) in an extractor (KSNP-B1180-240L, Hanyang Industrial, Korea). The mixture is then heated to 50 °C for 8 hours. Once the heating process is complete, we added 8% honey, 2% pear concentrate, 4% xylitol and 0.01% refined salt to the extracts, and stirred in persimmon vinegar at six different concentrations (0%, 2.5%, 5%, 7.5%, 10% and 12.5%).

2.2. Titratable acidity and pH assays

A pH meter (Metrohm 691, Switzerland) was used to determine the pH of samples during fermentation. The titratable acidity content was measured using a titratable acidity method. A 1 mL sample of persimmon vinegar was added to 1% phenolphthalein, and used as the indicator. The prepared sample was then added to 0.1 N NaOH, which was used as a neutralizing agent. The titratable acidity content was calculated from the amount of NaOH consumed until a color change occurred in the sample.

2.3. °Brix determination

The sugar content of vinegar samples was measured using a refractometer (IT, Atago Co., Japan).

2.4. Color assays

The color of the sample was measured using a spectrophotometer (UV-1201, Shimadzu Co., Japan), and expressed in terms of Hunter color values, such as L value, redness (a) and yellowness (b). The values of L, a and b on standard white plates were 99.22, -0.25 and -0.29, respectively.

2.5. Turbidity assays

Turbidity was measured by filtering vinegar (Whatman No. 1) using a spectrophotometer (UV-1201, Shimadzu Co., Japan) at 660 nm.

2.6. Assay for reducing sugar content

The reducing sugar content was measured using the dinitrosalicylic acid (DNS) method [6]. The samples (1 mL) were mixed with 1 mL of DNS solution, and 3 mL of distilled water was added to the mixture in a test tube. The mixture was heated in boiling water (100 °C) for 15 min and cooled to 25 ± 2 °C. A spectrophotometer (UV-1201, Shimadzu Co., Japan) was used to measure the absorbance at 546 nm. A standard glucose solution was prepared to obtain the standard curve. The R-value of the standard curve was 0.9957.

2.7. Assay for total tannin content

The total tannin content was measured using a colorimetric method according to the AOAC method [7]. Folin-Denis reagent (5 mL) was mixed with 1 mL of the sample and added to 5 mL of saturated Na₂CO₃ solution. The mixture was then incubated at 25 ± 2 °C for 30 min, and the absorbance was measured at 760 nm. The total tannin content was calculated using a standard curve.

2.8. Mineral content assays

Mineral analysis was performed using the method described by Martins et al. (2020) [8]. Inductively coupled plasma (ICP; Iris Intrepid, Thermo Elemental Co., UK) and iTEVA were used to

measure axial pressure. The parameters for the analysis were set as follows: 1.15 kW of power; 12 L min⁻¹ plasma flow rate; 0.5 L min⁻¹ auxiliary gas flow rate; 0.5 L min⁻¹ nebulizer flow rate; 30 s sample flush time; 2.0 mm torch injector tube i.d.; 3.0 mL min⁻¹ sample uptake rate; 35 rpm average speed of the pump; 1 s replicate reading time; and 15 s instrument stabilization delay. The experiments were performed in triplicate. Elemental determination was performed by selecting high-intensity and minimum interference of the corresponding wavelengths. The analytical lines of measurement were as follows: Ca 393.366(85) nm; Cu 324.754(103) nm; K 766.491(44) nm; Mg 285.313(117) nm; Mo 202.030(166) nm; Na 588.995(57) nm; and Zn 223.856(157) nm.

2.9. Free sugar content assays

A high-performance liquid chromatography coupled to a refraction index detector (HPLC-RI) with a water Sugar-Pak 1 column ($300 \times 6.5 \text{ mm}$, $10 \mu \text{m}$) was used to determine the sugar content of the samples. The flow rate was set at 0.60 mL min⁻¹, the injection volume was 20 µL and the column temperature was 90 °C. An isocratic solvent system consisting of ethylenediaminetetraacetic acid calcium disodium salt (EDTA; 50 mg L⁻¹) was used for 15 min. Calibration curves were constructed for each sugar standard (dissolved in water) for quantification. Each sample was aliquoted into a centrifuge tube, filtered with a 0.45-µM filter, and then injected into the HPLC-RI analyzer. All samples were analyzed three times.

2.10. Organic acid content assays

Organic content analysis was performed using the methods described by Pande and Akoh (2010) and Chen et al. (2006) [9,10]. Briefly, 2 ml of the sample was mixed with 10 mL of 1 M HCl, and the final volume was made up to 20 mL with 1 M HCl. The samples were flushed with nitrogen and centrifuged for 15 min (2000 rpm), heated for 30 min in a water bath at 90 °C, and then cooled to 25 °C. The supernatant was collected and filtered through a 0.45- μ m membrane filter. HPCL (Water 2414, Water Co., USA) was used to analyze and identify the organic acid content. The injection volume was set to 20 μ m, and the column temperature was maintained at 40 °C. An Agilent Zorbax Eclipse XDB-C18 column (3.5 μ m, 4.6 × 150 mm) was used for the chromatographic analysis. An isocratic mobile phase of 0.5% ammonium phosphate (pH adjusted to 2.8 with phosphoric acid) was used at a flow rate of 0.5 mL min⁻¹. The detection was performed at 214 nm.

2.11. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activity assays

This study used the method described by Choi et al. (1995) [11] with some modifications to determine ADH and ALDH activity. The *in vitro* activity of ADH was measured by NADH formation, and was determined spectrophotometrically at 340 nm using a UV/Vis spectrophotometer (UV-1201, Shimadzu Co., Japan). The first reaction was completed with the addition of 0.1 mL alcohol, 0.5 mL NAD aqueous solution (2 mg mL⁻¹), 0.1 mL persimmon vinegar and 0.01 M glycine-NaOH. Subsequently, the buffer solution (pH 8.8) was adjusted to a final volume of 1.8 mL. The mixture was allowed to react in a water bath at 25 °C for 10 min, and then 0.25 mL of ADH (10 units mL⁻¹) was added, and the absorbance at 340 nm was measured. Distilled water was added instead of the sample in the control reaction system. Hepos, which was used as a positive control, was purchased from a

pharmacy. It was diluted 1:1, according to the prescription, before use. The ADH activity was defined as the maximum absorbance at the end of the reaction and was compared with the maximum absorbance of the control. It is expressed as a ratio and was calculated as follows:

ADH activity =
$$(B/A) \times 100$$
, (1)

where:

A: the maximum absorbance of the control;

B: the maximum absorbance of the experimental group.

The ALDH activity was measured based on NADH production. This method was modified from that of Koivula and Koivusalo (1975) [12]. ALDH analysis was performed by preparing a solution containing 2.1 mL of distilled water, 0.3 mL of 1 M Tris HCl, 0.1 mL of 3 M KCl, the specified concentration of persimmon vinegar (0.1 mL), 0.1 mL of 20 mM NAD, 0.1 mL of 0.33 M 2-mercaptoethanol, and 0.1 M acetaldehyde (0.1 mL). The mixture was allowed to react in a water bath at 25 °C for 10 min with ALDH (1-unit mL⁻¹) (0.1 mL), and the absorbance was measured. ALDH activity was measured according to the formula used to calculate the ADH activity:

$$ALDH activity = (B/A) \times 100, \tag{2}$$

where:

A: the maximum absorbance of the control;

B: the maximum absorbance of the experimental group.

2.12. Statistical analysis

SAS (Statistical Analysis System, version 12.0) was used for all statistical data and variance analyses, and Duncan's multiple range tests were used to determine significant differences.

3. Results and discussion

3.1. Determination of pH, titratable acidity content, °Brix, turbidity, reducing sugar content and total tannin content

Table 1 shows the pH and titratable acidity content of the hangover relief drinks containing persimmon vinegar. The hangover relief drink without persimmon vinegar addition (0%) had the highest pH value (3.91 ± 0.01), and the lowest pH value (3.69 ± 0.01) was obtained with 12.5% persimmon vinegar addition. The pH decreased with increasing persimmon vinegar concentration. Another study found that the pH of persimmon peel vinegar was 3.64 [13]. In another study, pineapple waste vinegar attained a pH of 3 at the end of a 30-day acidification process [14].

Regular beverages sold on the market are measured at approximately 10 to 12% of °Brix, which is the most acceptable sugar concentration, and hangover-relieving drinks have similar °Brix values. The sugar content tended to decrease with the addition of persimmon vinegar. Similar to the result obtained in this study, it was found that the physicochemical properties of persimmon vinegar have been determined during fermentation; the titratable acidity content increased from 1.03 ± 0.01 to 2.47 ± 0.00 %, while °Brix values decreased from 11.1 ± 0.06 to 10.5 ± 0.06 %. These results indicate that persimmon fermentation is complete [15]. Turbidity is a critical quality attribute of liquid foods because consumers prefer less turbid products. The presence of suspended solids results in turbidity of the liquid medium [16]. Table 1 shows the chromaticity of the hangover relief drinks containing persimmon vinegar. The turbidity of the persimmon vinegar drinks increased as the quantity of persimmon vinegar increased. Chromaticity was highest (0.102 ± 0.002 %) when 12.5% vinegar was added, and lowest (0.060 ± 0.003 %) when no vinegar was added.

The reducing sugar content of the drinks prepared using varying concentrations of persimmon vinegar decreased with increasing persimmon vinegar concentration, a trend similar to that of the pH values. The highest reducing sugar content was 12.54 ± 0.38 mg/mL, obtained with the addition of 0% persimmon vinegar, and the lowest was 9.92 ± 0.43 mg/mL, obtained with the addition of 12.5% persimmon vinegar. In their study of balsamic vinegar, Laluo et al. [17] found that, among all samples, the total reducing sugar content decreased at the same time as the pH values decreased. Another study found that the reducing sugar content decreased from 7.68 \pm 0.02 g 100 mL⁻¹ to 2.64 \pm 0.01 g 100 mL⁻¹, and that the decline occurred slowly during alcohol fermentation of Zhenjiang aromatic vinegar [18].

The total tannin content of persimmon vinegar drinks tended to increase with the addition of persimmon vinegar; the highest value was 0.190 ± 0.004 mg/100 mL, and the lowest value was 0.091 ± 0.001 mg/100 mL. This result was supported by a previous study that reported that persimmon is rich in polyphenols, such as tannin, gallic acid and flavin-3-ols [19]. Zou et al. [15] also reported that acetic and alcoholic fermentation increased the bioactive properties and tannin concentration of persimmons. Therefore, the greater the amount of persimmon vinegar added, the higher the total tannin obtained.

Concentration of	pН	Titratable	°Brix (%)	Turbidity	Reducing	Total tannin
added persimmon		acidity (%)		(%)	sugar	(mg/100mL)
vingar (%)					(mg/mL)	
0	$3.91 \pm$	$1.03 \pm$	$11.1 \pm$	$0.060 \pm$	$12.54 \pm$	$0.091 \pm$
	$0.01^{d\ 1)}$	0.01 ^a	$0.06^{c1)}$	0.003 ^{a 1)}	0.38 ^{e 1)}	0.001 ^{a 1)}
2.5	$3.74 \pm$	$1.33 \pm$	$10.8 \pm$	$0.073 \pm$	$12.82 \pm$	$0.105 \pm$
	0.01 ^{ca}	0.01 ^b	0.06^{b}	0.004^{b}	0.43 ^e	0.004^{b}
5	3.71 ±	$1.60 \pm$	$10.8 \pm$	$0.080 \pm$	$12.06 \pm$	$0.116 \pm$
	0.01 ^b	0.01 ^c	0.06^{b}	0.004 ^c	0.17 ^{cd}	0.001 ^c
7.5	$3.72 \pm$	$1.83 \pm$	$10.7 \pm$	$0.091 \pm$	$11.77 \pm$	$0.146 \pm$
	0.01^{b}	0.02 ^d	0.06^{b}	0.003 ^d	0.21 ^{bc}	0.004 ^d
10	3.71 ±	2.17 ±	$10.5 \pm$	$0.097 \pm$	$11.31 \pm$	$0.170 \pm$
	0.01^{b}	0.01 ^d	0.00^{a}	0.002 ^e	0.11 ^b	0.006 ^e
12.5	$3.69 \pm$	$2.47 \pm$	$10.5 \pm$	$0.102 \pm$	9.92 ± 0.43^a	$0.190 \pm$
	0.01^{a}	0.00^{e}	0.06^{a}	0.002 ^e		0.004^{f}

Table 1. Effects of persimmon vinegar addition on pH, titratable acidity content, °Brix, turbidity, reducing sugar content and total tannin content of alcohol relief drink.

1) Values are mean \pm standard deviation; a–f) Values with different letters in the same column are significantly different (P < 0.05).

3.2. Color

Table 2 shows the chromaticity measurement results of L (lightness), a (redness) and b (yellowness) values for the hangover relief drinks. The highest L value was 24.64 ± 0.02 , obtained for 0% persimmon vinegar addition; the lowest value was 23.49 ± 0.05 obtained for 12.5% persimmon vinegar addition. The L value decreased as persimmon vinegar concentration increased. Different trends were observed for these values. The lowest and highest a values were 0.30 ± 0.06 and 0.62 ± 0.05 , respectively, obtained from the addition of 0 and 12.5% persimmon vinegar. In contrast, the b value was similar in all six treatments, within the range of 2.65 ± 0.01 and 2.60 ± 0.06 . Color is an essential factor related to the sensory properties of a product [20]. The color change, especially on L values, depends on the persimmon vinegar concentration, which influences the tannin content.

Concentration of added	L	a	b
persimmon vinegar (%)			
0	$24.64 \pm 0.02^{f1)}$	$0.30\pm0.06^{\rm a}$	2.65 ± 0.01
2.5	$24.34\pm0.02^{\text{e}}$	$0.36\pm0.06^{\text{b}}$	2.63 ± 0.06
5	$24.04\pm0.04^{\text{d}}$	$0.46\pm0.02^{\rm c}$	2.60 ± 0.04
7.5	$23.82\pm0.01^{\text{c}}$	$0.53\pm0.02^{\rm c}$	2.63 ± 0.03
10	$23.61\pm0.02^{\text{b}}$	$0.62\pm0.04^{\text{d}}$	2.61 ± 0.06
12.5	$23.49\pm0.05^{\rm a}$	$0.62\pm0.05^{\text{d}}$	2.60 ± 0.06

Table 2. Effects of persimmon vinegar addition on color of alcohol relief drink.

1) Values are mean \pm standard deviation; a–f) Values with different letters in the same column are significantly different (P < 0.05).

3.3. Mineral content

Table 3 shows the results of inorganic substances in hangover-relieving beverages containing persimmon vinegar. The lowest measured value of Ca was 14.74 ± 0.23 ml/L, and the addition of 12.5%vinegar resulted in the highest value for Ca ($21.37 \pm 2.15 \text{ ml/L}$). The Cu content increased with the addition of 7.5% persimmon vinegar, whereas the addition of 7.5% or more vinegar resulted in a decrease in Cu content, with the addition of 10% vinegar showing the lowest value (0.098 \pm 0.039 ml/L). The K content increased as the amount of persimmon vinegar increased. The 12.5% addition showed the highest value of 208.60 ± 3.42 ml/L. Mg and Zn content also showed trends similar to K content, with the 12.5% addition showing the highest value of Mg (17.21 ± 0.74 ml/L) and Zn (0.386 \pm 0.102 ml/L). The lowest values for these two minerals were found for 0% persimmon vinegar addition at 8.03 \pm 0.25 ml/L and 0.274 \pm 0.005 ml/L, respectively. For Mo and Na, the 0% treatment resulted in values of 0.0110 ± 0.002 ml/L and 116.16 ± 0.94 ml/L, respectively. According to the above results, the mineral content of the hangover relief drink with added persimmon vinegar showed increasing mineral content for Ca, K, Mg and Zn as the amount of persimmon vinegar, liquid rich in inorganic content, increased. The results also showed that the most abundant minerals in persimmon vinegar were Ca, K and Na. Similarly, Na, K and Ca are the most abundant minerals present in vinegar [16]. Alcohol can interfere with the absorption and metabolism of nutrients in the body, including minerals, such as magnesium, potassium and sodium. These minerals are important for maintaining electrolyte balance and hydration, and their deficiency can contribute to hangover

symptoms. Therefore, it is possible that beverages with higher mineral content may help to alleviate hangover symptoms by replacing some of the minerals that are lost during alcohol metabolism.

Mineral content	Persimmon vinegar (%)						
mg/L	0	2.5	5.0	7.5	10.0	12.5	
Са	$14.740~\pm$	$15.390 \pm$	$15.410 \pm$	$16.760 \pm$	$17.780 \pm$	$21.370 \pm$	
	$0.230^{b1)}$	0.33 ^a	0.69 ^a	0.620 ^b	0.74 ^b	2.150 ^c	
Cu	$0.122 \pm$	$0.127 \pm$	$0.132 \pm$	$0.289 \pm$	$0.098 \pm$	$0.112 \pm$	
	0.092	0.056	0.025	0.317	0.039	0.054	
Κ	$49.860 \pm$	$90.610 \pm$	$114.130 \pm$	$145.500 \pm$	$183.910 \pm$	$208.600 \pm$	
	1.45 ^a	1.41 ^b	2.76 ^d	7.020 ^d	7.070 ^e	3.420^{f}	
Mg	$8.030 \pm$	$8.740 \pm$	$9.900 \pm$	$11.300 \pm$	$13.830 \pm$	$17.210 \pm$	
	0.250 ^a	0.180 ^a	0.28 ^c	0.620 ^c	0.620 ^d	0.740^{e}	
Мо	$0.011 \; \pm$	$0.008~\pm$	$0.007 \pm$	$0.006 \pm$	$0.006 \pm$	$0.006 \pm$	
	0.002°	0.002^{b}	0.000^{ab}	0.002^{ab}	0.001^{ab}	0.002^{a}	
Na	$116.15 \pm$	$104.38 \pm$	$104.290 \pm$	$106.660 \pm$	$109.300 \pm$	$103.380 \pm$	
	0.940 ^c	2.03 ^a	3.02 ^{ab}	4.06^{ab}	2.430 ^b	1.490 ^a	
Zn	$0.274~\pm$	$0.285 \pm$	$0.300 \pm$	$0.329 \pm$	$0.367 \pm$	$0.386\pm$	
	0.005^{a}	0.000^{a}	0.001^{ab}	0.001^{ab}	0.003 ^{bc}	0.102 ^c	

Table 3. Effects of persimmon vinegar addition on mineral content of alcohol relief drink.

1) Values are mean \pm standard deviation; a–f) Values with different letters in the same column are significantly different (P < 0.05).

3.4. Free sugar content

Table 4 shows results of the free sugar content of hangover relief drinks containing persimmon vinegar. The free sugar content of the hangover relief drink showed a significant increase in the order of fructose, glucose and maltose. The 0% persimmon addition treatment showed the highest value of Fructose (334.60 mg/100 mL), whereas the 12.5% addition treatment showed the lowest value. Xylose and lactose were not detected. For sucrose, the lowest value in the control was 3.86 mg/100 mL, and the highest value was obtained at the 12.5% addition (8.43 mg/100 mL). The free sugar content tended to decrease as the amount of vinegar added increased; however, sucrose tended to increase slightly. The sucrose content may increase because of the fermentation process of persimmon vinegar production due to the conversion of the sucrose into other compounds. This can occur because persimmons are a source of sucrose, and the microorganisms present in the fermentation process may consume and convert some of this sucrose into other compounds. Thus, the more persimmon vinegar added, the higher the sucrose content.

3.5. Organic acid content

Table 5 shows content of organic acids in hangover relief drinks supplemented with persimmon vinegar. The drink had organic acid contents in the order of acetic, lactic and oxalic acid, with acetic acid found to be the most abundant. In the case of acetic acid, as the amount of vinegar added increased, a significant increase was observed. For the drink without persimmon vinegar, acetic acid

concentration was 49.09 mg/100 mL, and the addition of 12.5% persimmon vinegar resulted in an increase in acetic acid concentration to 217.01 mg/100 mL. As previously reported, ingestion of persimmon vinegar, in which the main ingredients are acetic acid and other organic acids, promotes appetite and hinders glycolysis [21].

Free sugar	Concentration of added persimmon vinegar (%)						
content	0	2.5	5.0	7.5	10.0	12.5	
(mg/100 mL)							
Xylose	ND	ND	ND	ND	ND	ND	
Fructose	334.6	319.93	318.2	315.29	311.29	276.17	
Glucose	299.73	280.58	273.41	272.9	268.62	242.37	
Sucrose	3.86	6.67	7.8	8.12	8.34	8.43	
Maltose	40.96	34.96	34.34	33.7	33.23	32.4	
Lactose	ND	ND	ND	ND	ND	ND	

Table 4. Effects of persimmon vinegar addition on free sugar of alcohol relief drink.

ND: Not detected.

Table 5. Effects of persimmon vinegar addition on organic acid of alcohol relief drink.

Organic acid	Concentra	Concentration of added persimmon vinegar (%)						
content	0	2.5	5.0	7.5	10.0	12.5		
(mg/100 mL)								
Oxalic acid	41.18	42.07	41.61	44.23	44.57	45.82		
Tartaric acid	ND	ND	ND	ND	ND	ND		
Malic acid	25.84	25.85	26.63	27.34	28.52	29.24		
Lactic acid	42.79	43.12	43.52	49.71	54.91	55.94		
Acetic acid	49.09	86.35	127.45	164.39	203.76	217.01		
Citric acid	29.65	29.28	30.21	28	30.4	29.45		
Succinic acid	11.6	11.12	11.14	11.15	11.29	11.42		

ND: Not detected.

In the case of oxalic acid and lactic acid, the values gradually increased as the amount of vinegar added increased, whereas in the case of malic, citric and succinic acids, similar values were observed in each treatment, indicating no significant difference. Malic acid is characterized by its taste and acts as a flavor blender to create a smoother and more natural flavor [22]. Liu et al. [23] reported that malic acid significantly increased to 29.94% during persimmon wine production. Tartaric acid was not present in any of the tested samples. Overall, as the amount of vinegar was increased, the organic acid content gradually increased. Ren et al. [24] detected acetic, lactic, quinic, tartaric, propanedioic, malic, succinic and citric acids as the organic acids in persimmon vinegar. The highest succinic acid content was 11.60% when persimmon vinegar was not used. Succinic acid is a common metabolite produced by yeast during fermentation [23].

3.6. ADH and ALDH activities

Figure 1 shows the results of measuring the activities of ADH and ALDH in hangover relief drinks containing persimmon vinegar. The relative ADH activity increased as vinegar concentration increased. For 0, 2.5, 5, 7.5, 10 and 12.5% persimmon vinegar, the values were 124.07, 130.86, 138.27, 147.53, 152.88 and 160.91%, respectively. The ALDH activity increased as the percentage of added vinegar increased (96.97, 98.66, 101.51, 106.72, 113.78 and 117.14% for 0, 2.5, 5, 7.5, 10 and 12.5% persimmon vinegar addition, respectively).

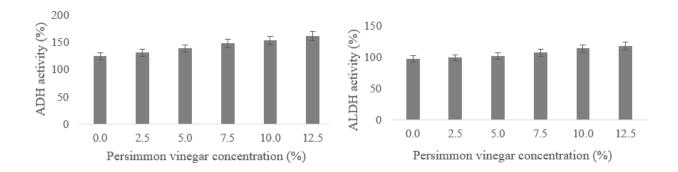


Figure 1. Effects of persimmon vinegar addition on alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities in the alcohol relief drink.

Kim et al. [25–27] reported that consuming a sports drink containing persimmon vinegar resulted in the activation of oxygen transport during exercise, and improved recovery from exercise-induced fatigue. The drink with the highest ADH and ALDH activities was 12.5% persimmon vinegar. The same sample also contained the highest amount of acetic acid. Studies have shown that acetic acid can increase the activity of ADH and ALDH in animals and in cell cultures. For example, a study in rats found that acetic acid increased the activity of ADH in the liver, leading to a reduction in blood alcohol levels [28]. In addition, ALDH was strongly correlated with the average acidification rate. Furthermore, alcohol metabolism is accelerated by increasing the activity of alcohol-metabolizing enzymes, activating the anti-oxidative enzyme system against oxidative stress, and decreasing fat accumulation [29]. Therefore, persimmon fruit and leaf extracts might have the ability to improve alcohol metabolism and liver lipid profiles because they contain antioxidant components, such as flavones and phenolics. A study of the antioxidant activity of persimmon vinegar found that the highest activity in this test and inhibition rate was 89.7% [30]. In addition, the total antioxidant capacity of the total flavonoid extract of persimmon leaves is close to that of rutin, at a dose of 400 mg/mL [31].

4. Conclusions

Preparing alcoholic beverages with persimmon vinegar affected the physical aspects of the alcohol relief drink. Higher persimmon vinegar resulted in a lower pH, °Brix and reducing sugar, but higher titratable acidity, turbidity and total tannin content in the product. However, adding more than 7.5% persimmon vinegar did not yield substantially different pH, °Brix and reducing sugar values. The pH, °Brix and reducing sugar values of 7.5% persimmon addition were 3.72 ± 0.01 , 10.7 ± 0.00

and 11.77 ± 0.21 , respectively. The 7.5% persimmon vinegar addition-product had a titratable acidity content of 1.83 ± 0.02 , turbidity of 0.091 ± 0.003 and total tannin of 0.146 ± 0.004 . There was no significant difference in the titratable acidity content between the 7.5 and 10% persimmon addition-products. The results also showed that the L value decreased drastically with the addition of 7.5% persimmon vinegar. Additionally, high concentrations of persimmon vinegar reduced fructose, glucose and maltose content. However, the sucrose content of the products increased significantly when 7.5% persimmon vinegar was added. Generally, Ca, K and Na have the highest mineral contents in drinks containing persimmon vinegar. In addition, the amounts of oxalic, malic, lactic, acetic and succinic acids increased. However, 7.5% persimmon vinegar had the lowest citric acid content, and increased ADH and ALDH activities were observed as the percentage of persimmon vinegar to alcohol hangover relief drinks had a greater impact on the physicochemical characteristics of the product. This finding indicates that the optimization usage of local food ingredients, such as persimmon vinegar and herbals selected ingredients in the formulation of alcohol hangover relief drinks, is worth studying further.

Conflict of interest

We declare no conflicts of interest in this paper.

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