



Development of Fermented Banana Vinegar: Chemical Characterization and Antioxidant Activity

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Abstract

This study was carried out to examine the chemical properties, antioxidant activities and sensory scores of banana vinegar produced from four banana cultivars, namely ‘Khai Pra Tabong’, ‘Nak’, ‘Hin’, and ‘Phama Heak Kuk’. The initial soluble solid contents in the banana juice were adjusted to 25 °Brix before taking to fermentation. Alcoholic fermentation was conducted using *Saccharomyces cerevisiae* as the inoculant while *Acetobacter pasteurianus* was used for acetous fermentation. As observed, all samples during the alcoholic fermentation the levels of soluble solids decreased continuously and the level of alcohol were found to increase at the end of fermentation process. Notably, the wine produced from ‘Phama Heak Kuk’ cultivar exhibited the highest level of alcohol (9.54 %) and exhibited the highest levels of antioxidant activity (87.04 %). Similar results were observed for all samples during the acetous fermentation, in which the level of alcohol dropped continuously and the levels of acetic acid were noted to elevate at the end of the fermentation process. The highest levels of acetic acid (3.49 %) was detected in the vinegars produced from ‘Phama Heak Kuk’ cultivar while those produced from ‘Khai Pra Tabong’ cultivar exhibited the highest levels of antioxidant activity (80.59 %). Sensory evaluation based on the 9-point hedonic scales showed that the vinegars produced from ‘Khai Pra Tabong’ cultivar showed the highest overall acceptability with an average score of 8.13, equivalent to the hedonic scale of 9, which indicated a high pleasant level of the vinegar preference of the consumers.

Introduction

Due to its availability in several different varieties in every country, vinegar represents one of the most

widely used seasonings in the world (Jo et al., 2013). In addition to being primarily used as food seasoning, vinegar plays an important role in the production of food

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products since it is applied in a wide variety of products, including sauces, ketchups and mayonnaise (Ho et al., 2017). Moreover, vinegar has long been used in the treatment of many common ailments with claims of anti-infective, antitumor, and hyperglycemic properties (Johnston & Gaas, 2006; Wongsudarak & Nunium, 2013). The production of vinegar is in general low in costs due to the fact that inexpensive raw materials like by-products from food processing, fruit waste, substandard fruit and agricultural surpluses are utilized (Solieri & Giudici, 2009). The beneficial effects of vinegar might be due to bioactive substances such as amino acids, organic acids or phenolic compounds derived from its raw materials (Budak et al., 2014; Ghosh et al., 2016). Moreover, the bioactive compounds in vinegars can be produced and/or increased through the overall vinegar fermentation process (Solieri & Giudici, 2009), where phenolic compounds are transformed into new antioxidative molecules (Shahidi et al., 2008). Additionally, the aroma and flavor of vinegars impacting on consumer acceptance is influenced by the raw materials used, the compounds formed during the fermentation process, and the fermentation type used (Callejon et al., 2008; Liang et al., 2016; Morales et al., 2002; Ubeda et al., 2012). Recently, the demand for fruit vinegars has increased due to their reputation as health food products, which help 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 low density lipoproteins (LDLs), among other benefits (Chen et al., 2017).

Musa spp. Comprising dessert bananas and plantains, were among the world leading fruit crops as source of energy in the diet of people living in humid tropical regions (Sudhanyaratana

et al., 2016). Banana has firm pulp when the fruit is not ripe and soft pulp during maturation. It is known that dessert banana pulp and peel contains some secondary metabolites in their composition, e.g. catecholamines, phenolics, and carotenoid compounds as well as pyridoxine. Many of banana's volatile compounds such as ester and alcohols play an important role in the aromatic properties of banana. (Pereira & Maraschin, 2015). According to Coelho et al. (2017), who studied the chemical composition and antioxidant activity of banana vinegar produced from fruit concentrates, the results revealed the total acidity of banana vinegar (5.4 %) and the antioxidant activity by Frap (3.7 mmol Fe₂SO₄/L)

For this purpose, this study was carried out to

compare the chemical properties, antioxidant activities and sensory scores of the banana vinegars produced via a two-stage fermentation process from four cultivars, namely 'Khai Pra Tabong', 'Nak', 'Hin', and 'Phama Heak Kuk'. In this context, chemical properties were assessed in terms of alcohol contents, glucose and fructose contents, and acetic acid contents. Antioxidant activities were determined by DPPH radical assays and total phenolic contents. Sensory evaluation was performed based on the 9-point hedonic scale in order to determine the consumers preference.

Materials and methods

1. Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was purchased from Sigma-Aldrich (Steinheim, Germany). Iron (II) sulfate, iron (III) chloride 6-hydrate and gallic acid standard were supplied by Fluka (Buchs, Switzerland). 2,4,6-tri-2-pyridyl-2-triazine (TPTZ) and sodium acetate 3-hydrate were obtained from HiMedia Laboratories (Mumbai, India). Folin-ciocalteau reagent was from Merck (Darmstadt, Germany) and sodium carbonate (anhydrous) from Univar (Downers Grove, IL, USA). All other chemicals and solvents were purchased from local manufacturers. Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, USA).

2. Raw materials and fermentation

Banana fruits of four cultivars, namely 'Khai Pra Tabong' (The short stem is curved, thick peel. When ripe had yellow color, orange flesh and sweet flavor), 'Nak' (The raw banana is bright red, the ripe banana is an orange-red color), 'Hin' (Yellow thick peel, creamy white pulp with a sweet flavor), and 'Phama Heak Kuk' (The raw banana is a dark green color, the ripe fruit is an orange-red color), was used for the production of banana vinegars via a two-stage (alcoholic and acetous) fermentation process. Banana fruits of each cultivar were crushed and mixed with water at a ratio of 1:1 to prepare banana juice. After adjustment of the pH to 4.5 using 5% acetic acid and sugar content up to 25° Brix.

3. Banana vinegar production

Amylase enzyme from Mc-Zyme S.P. Corporation was applied in order to degrade the starch still present in the banana puree for 24 hours at 30°C. The banana juice was pasteurized for 30 min at 60°C. Alcoholic fermentation was conducted for 5 days at room temperature under static conditions in plastic vessels

containing 2 L of the banana juice inoculated with wine yeast, *Saccharomyces cerevisiae*, (Wine & Scientific Equipment Ltd., Part) at a ratio of 0.75% (v/v). 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 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, modified from the method of Coelho et al (2017), the alcohol content of the obtained wine was adjusted to 6 %. Acetous fermentation was performed for 15 days under the aforementioned conditions in glass vessels containing 135 mL of the banana wine inoculated with *Acetobacter pasteurianus* TISTR 521 at a ratio of 10% (v/v). Sampling was performed at given time-points to collect the two-stage fermented banana vinegars by allowing them to settle in microtube and stored at 4°C before the analyses.

4. Chemical analysis

Analysis of alcohol, acetic acid, glucose and fructose contents modified from the method of Aguiar et al. (2005). The analysis was performed on a Shimadzu HPLC-RID system (Shimadzu, Japan) consisting of Shimadzu LC-20AD pumps and 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). H₂SO₄ (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 45°C.

Total soluble solids values of the wine were measured using an AllA France refractometer (AllA France, France) calibrated with distilled water. The values were expressed as oBrix.

5. Antioxidant activity

Antioxidant activities of the vinegars were evaluated by DPPH radical assay (Brand-Williams et al., 1995) in which 2,2-diphenyl-1-picrylhydrazyl hydrate (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 the percentage of the DPPH radical elimination effect of vitamin C. Control solutions were

prepared by dissolving 0.004 g of DPPH in 95% ethanol, followed by adjustment of the solutions to a final volume of 100 mL. DPPH radical scavenging capacity (RSC) was calculated using the equation $\%RSC = (A_c - A_s/A_c) \times 100$, where A_c and A_s denote the absorbance of control and sample, respectively.

6. Total phenolic content analysis

Total phenolic contents of the banana vinegars were determined using Folin-Ciocalteu reagent as described by Singleton et al (1999). 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% Na₂CO₃ 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/mL).

7. Sensory evaluation

200 g of the banana vinegars were mixed with 150 g of honey and 150 g of water to make drinking vinegars and the obtained drinking vinegars were subjected to the sensory evaluation based on the 9-point hedonic scale by using 30 untrained panelists for 5 attributes (sweet, color, odor, taste and overall acceptance) with the scale 9 representing like extremely, 5 representing neither like nor dislike and 1 representing dislike extremely.

8. Statistical analysis

A randomized block design, with three replicates and four samples per replicate, was used to compare the chemical properties, antioxidant activities and sensory evaluation of the banana vinegars produced from four banana cultivars. The results are expressed as the mean ± standard deviation (SD) and data were analyzed using 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.

Results and discussion

1. Chemical properties of the banana wines and vinegars

The banana wines produced from four banana cultivars 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 Fig. 1A. It was observed that at the end of the fermentation, high alcohol content was detected

in all the banana wines, indicating that sugars in the banana juice were rapidly converted to alcohol. The banana wine produced from 'Phama Heak Kuk' cultivar contained the highest alcohol content of 9.54 %, which was higher than that (9.27%) detected in the lychee wines produced in an earlier study (Chen & Liu, 2016). As given in Fig. 1B, Glucose was rapidly utilized during the production of the banana wine as observed for all samples, with the most rapidly utilized glucose observed after 1 day of the fermentation in 'Nak' cultivar. Notably, glucose was completely depleted in all wine samples after 4 days of the fermentation. Fructose was likely utilized more slowly as compared to glucose (Fig. 1C). The most rapidly utilized fructose were observed in the banana wine produced from 'Phama Heak Kuk', Khai Pra Tabong and Hin cultivar which was decreased more than Hin in 3 days of the fermentation. The rapid utilization of glucose and fructose and the consequent increase in the levels of alcohol confirmed that the yeast dominated the fermentation, which was supported by an earlier study (Taniyasuri et al., 2016) which elucidated the rapid utilization of glucose and fructose in the production of durian wine, in which at the end of the fermentation

fructose was completely depleted while glucose remained at 0.046 g/100 mL. In Fig.1D, total soluble solid of 4 wine were adjust to 25 oBrix after fermenting in 5 days, the results showed that Nak wine (15.47 °Brix) had greater TSS than Khai Pra Tabong (12 °Brix). From the experiment, it was found that the alcohol content in Khai Pra Tabong wine had greater values than Nak wine because yeast had the ability to consume TSS in the Khai Pra Tabong effectively. During the 15-day acetous fermentation process, Oxidative fermentation is a fermentation process caused by bacteria that requires oxygen to respire at the cellular level (Pongdam, 2017). The banana vinegars produced from the four banana wines using *A. pasteurianus* were analyzed for their chemical compositions, and the results are given in Fig. 2. All the banana vinegars showed a significant decrease in the alcohol content as it was converted to acetic acid by acetic acid bacteria, which was consistent with the increased acetic acid content. However, the alcohols were not completely depleted, at the end of acetous fermentation the vinegar produced from 'Nak' cultivar contained the highest alcohol content of 0.81 % while that produced from 'Khai Pra Tabong' cultivar had the

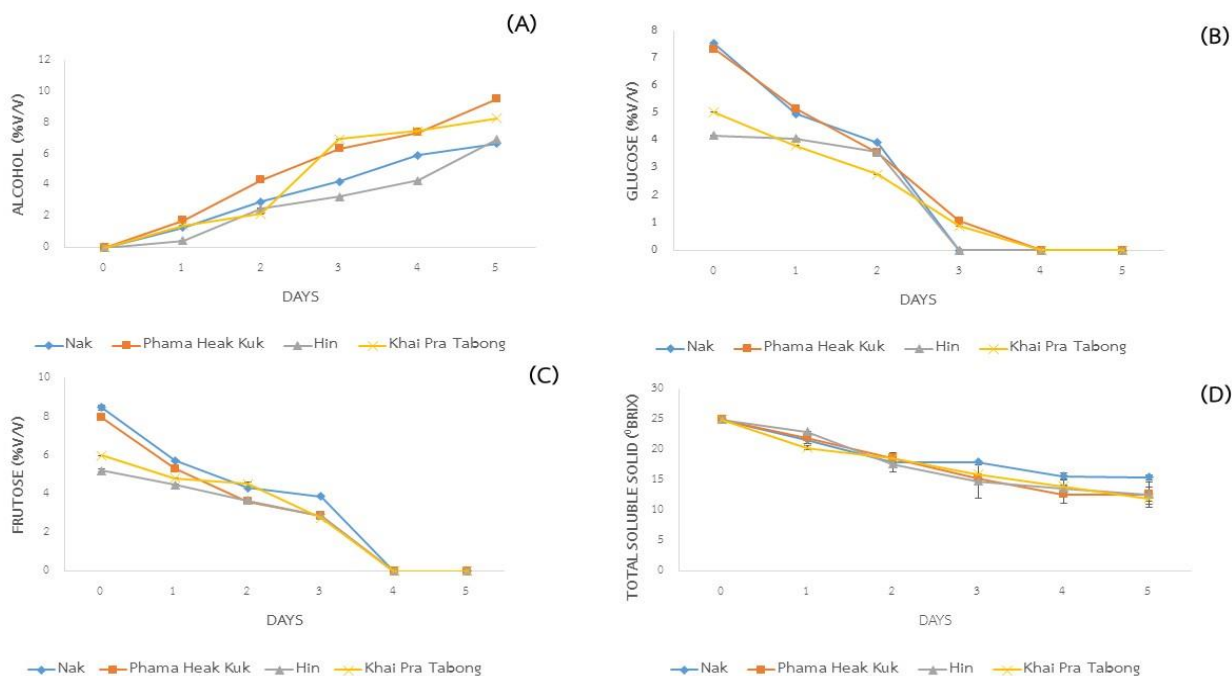


Fig. 1 Physicochemical properties of banana wine during a 5-days fermentation process alcohol (A) glucose (B) fructose (C) and total soluble solid (D)

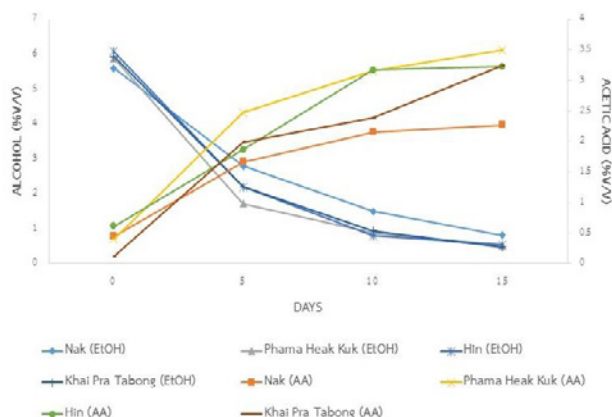


Fig. 2 Physicochemical properties of banana vinegar from banana 4 cultivars during a 15-day fermentation process

lowest alcohol content of 0.47 %. In an earlier study (Li et al., 2014) which elucidated that the alcohol content in the *Hericium erinaceus* vinegar was 0% after 9 days of acetic fermentation. Regarding the acetous fermentation, at the end of a 15-day acetous fermentation process, acetic acid content was found to range from 2.27% to 3.49%, with the highest value of 3.49% observed in the banana vinegar produced from 'Phama Heak Kuk' cultivar and the lowest of 2.27 % produced from 'Nak' cultivar which was much greater than that obtained in a previous study (Li et al., 2014), in which an acetic acid content of 21.56 mg/mL was detected in the *H. erinaceus* vinegar after 9 days of acetous fermentation. The acetic acid content in vinegar was not up to the standard of fermented vinegar (least 4% acetic acid), this could be caused by the same condition in fermentation had the problem. Ex. The ability of survival of acetic acid bacteria or the deficiency of aeration in fermentation.

2. Total phenolic contents and antioxidant activities

The levels of antioxidant activities of the banana vinegars are presented in Table 1. The results showed that the banana wine derived from 'Phama Heak Kuk' and 'Khai Pra Tabong' cultivar exhibited the highest antioxidant activity of 87.04% and 86.94%, which was greater than that produced from citrus fruit (36.8 ± 0.09%) (Chen et al., 2017). On the other hand, the vinegar produced from 'Khai Pra Tabong' cultivar was observed to exhibit the highest antioxidant activity of 80.59 ± 2.30%, which was much greater than that detected in the purple sweet potato *makgeolli* vinegar (67.63 ± 0.17%) (Chun et al., 2014). The levels of total

phenolic contents detected in the banana vinegars produced from different banana cultivars via a two-stage fermentation process are given in Table 2. It was noted that the banana wine derived from 'Nak' cultivar contained the highest levels (229.30 ± 0.59 mg/L) of total phenolics. Similar results were observed for the banana wine produced from the same cultivar, in which the vinegar measured at the end of acetous fermentation exhibited the highest total phenolic content of 243.98 ± 3.35 mg/L, which was much greater than that detected in the purple sweet potato *makgeolli* vinegar (24.73 ± 0.04 mg/L) (Chun et al., 2014). The antioxidant activity and total phenolic content were decreased in vinegar, the results were in agreement with an earlier study of Towantakavanit et al. (2011) which demonstrated that the drop in total phenol level could be due to fermentation process condensation and polymerization reactions as well as the formation of oxidative products and precipitations occur.

Table 1 Antioxidant activities of the four banana vinegars produced via a two-stage fermentation process

Cultivars	DPPH (% inhibition)	
	Wine	Vinegar
Nak	52.48 ± 1.87 ^c	47.44 ± 5.03 ^c
Phama Heak Kuk	87.04 ± 0.00 ^a	68.04 ± 1.49 ^b
Hin	72.08 ± 0.05 ^b	69.30 ± 8.09 ^b
Khai Pra Tabong	86.94 ± 0.05 ^a	80.59 ± 2.30 ^a

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

Table 2 Total phenolic contents of the four banana vinegars produced via a two-stage fermentation process

Cultivars	Total phenolic content (mg/L)	
	Wine	Vinegar
Nak	229.30 ± 0.59 ^a	243.98 ± 3.35 ^a
Phama Heak Kuk	126.28 ± 0.21 ^d	102.23 ± 0.69 ^d
Hin	214.57 ± 0.23 ^b	198.26 ± 1.61 ^b
Khai Pra Tabong	166.77 ± 0.47 ^c	115.92 ± 9.40 ^c

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

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 banana cultivars and honey, are depicted in Table 3. The results showed that significant ($p < 0.05$) differences in sweet odor sour and

Table 3 Sensory scores of the drinking vinegars blended from the four fermented banana vinegars

Cultivars	Sweet	Color	Odor	Sour	Overall acceptability
Nak	7.17 ± 1.90 ^{ab}	6.57 ± 1.68 ^{ns}	5.97 ± 1.52 ^{ab}	6.63 ± 1.59 ^b	7.57 ± 1.55 ^{ab}
Phama Heak Kuk	6.63 ± 1.47 ^b	6.77 ± 1.3 ^{ns}	5.80 ± 1.69 ^b	6.60 ± 1.38 ^b	6.93 ± 1.31 ^{bc}
Hin	7.87 ± 0.97 ^a	7.30 ± 1.34 ^{ns}	6.87 ± 1.89 ^a	7.63 ± 1.13 ^a	8.13 ± 1.14 ^a
Khai Pra Tabong	6.37 ± 1.90 ^b	6.50 ± 1.63 ^{ns}	5.70 ± 1.74 ^b	6.73 ± 1.91 ^b	6.70 ± 1.66 ^c

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

overall acceptability were observed among the drinking vinegars produced from different banana cultivars. The drinking vinegar produced from 'Hin' cultivar displayed the highest level of consumers' preference, with the mean overall acceptability score of 8.13 ± 1.14 , which was equivalent to the hedonic scale of 9. In our study, the high levels of consumers' preference of drinking banana vinegars might be attributed to the addition of honey, which was well supported by an earlier study (Marrufo-Curtido et al., 2015) which elucidated that the addition of dietary fiber derived from citrus fruits enhanced the phenolic and volatile profile as well as the judges' preference of the vinegar.

Conclusion

This study was conducted in order to compare the levels of acetic acid, total phenolics, antioxidants and consumers preference of the banana vinegars produced from four banana cultivars via a two-stage fermentation process. The results show that the vinegars produced from 'Phama Heak Kuk' cultivar exhibited the highest level of acetic acid (3.49%) while those produced from 'Khai Pra Tabong' cultivar displayed the highest antioxidant activities (80.59%) measured by means of DPPH radical assay. Meanwhile, the vinegars produced from 'Nak' cultivar were observed to have the highest total phenolics (243.98 mg/L). Sensory evaluation based on the 9-point hedonic scale using untrained panelists showed that the drinking vinegars made from 'Hin' cultivar had the highest overall preference (8.13).

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