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Research article

Physicochemical properties and antioxidant activity of banana vinegar produced using one-stage and two-stage fermentation

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Abstract

Fruit and vegetable waste has been widely utilized as a low-cost natural source for vinegar production. In this study, the pulp of banana (*Musa acuminata* 'Gros Michel') cv. 'Hom Thong' was utilized to produce vinegar using one-stage (acetous) and two-stage (alcoholic and acetous) fermentation and the obtained vinegars were assessed for their physicochemical properties, antioxidant activities and polyphenolic contents. It was found that the one-stage fermentation yielded greater levels of acetic acid (5.83% volume per volume; v/v) than two-stage fermentation (5.13% v/v). The 2,2-diphenyl-1-picrylhydrazyl hydrate and ferric reducing/antioxidant power assays, respectively, indicated that the antioxidant activity of the one-stage fermented vinegars (80.27% and 1274 µM Fe(II)/L) was greater than that of the two-stage fermented vinegars (73.20%, 1001 µM Fe(II)/L). In a similar manner, the polyphenolic content of the one-stage fermented vinegars (92.67 µg/mL) was higher than that of the two-stage fermented vinegars (68.01 µg/mL). The color parameters (*L*, a*, b**) only showed significant differences in *a** for the color change of the vinegars aged during days 0–100 for the one- and two-stage fermented vinegars. Moreover, it was observed that the one-stage fermented vinegars had higher levels of acetic acid, antioxidant activity and polyphenolics contents than the two-stage fermented vinegars. The results suggested that the use of banana pulp to produce vinegar via one-stage fermentation is a good choice to retain the bioactive compounds.

Introduction

Vinegar represents one of the most popular food items that people currently consume to promote health and reduce healthcare costs (Hashimoto et al., 2013) due to its functional therapeutic activities, which include antioxidant activity (Verzelloni et al., 2007; Ozturk et al., 2015; Yusoff et al., 2015) anti-infective properties (Wang et al., 2012b; Ozturk et al., 2015), anticancer and antitumor activities (Nanda et al., 2004; Johnston and Gaas, 2006; Baba et al., 2013) anti-obesity and anti-diabetic activity (Johnston et al., 2013; Yusoff et al., 2015) and lipid lowering effects (Lee et al., 2013; Park et al., 2014). In general, vinegar is produced based on a double fermentation process, namely, alcohol fermentation and acetic acid fermentation (Ozturk et al., 2015; Ubeda et al., 2016). Furthermore, vinegar production can be achieved using a one-stage fermentation process, in which absolute alcohol is added to fruit puree prior to the fermentation by acetic acid bacteria and this helps to reduce the production time as well as the cost of production (Yin et al., 2018). However, the

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phytochemical properties of the vinegars obtained from these two processes might be different and should therefore be studied.

Due to the health benefits proven by many reports (Sakanaka and Ishihara, 2008; Chen et al., 2011; Li et al., 2014; Wang et al., 2012a), there has been a growing demand for fruit vinegars, which are sold as a health food (Ou and Chang, 2009). The literature has revealed that the health benefits of fruit vinegars are profoundly dependent on their antioxidant activities (Alonso et al., 2004; Dávalos et al., 2005). As phenolic compounds play a critical role in the antioxidant activities of fruit made into vinegar (Wang et al., 2012a; Barnaba et al., 2015) fruits that are rich in phenolic compounds are considered as a potential substrate for vinegar production. Bananas are one of the important fruits that contain potentially bioactive compounds and are a great source of phenolic compounds such as phenolic acids and flavonoids (Nguyen et al., 2003; Tsamo et al., 2015). Banana pulp and peel contains various phenolic compounds, such as gallic acid, catechin, epicatechin, tannins and anthocyanins (Singh et al., 2016), and it has been well documented that the total phenolic compounds are more abundant in the peel than in the pulp (Ummarat et al., 2011). Thus, the current study was carried out to produce fruit vinegars using one-stage and two-stage fermentation utilizing 'Hom Thong' bananas as the substrate. The physicochemical properties and antioxidant activities of the one-stage and two-stage fermented vinegars were compared. In this context, the physicochemical properties were assessed in terms of acetic acid content, pH, total soluble solid, alcohol content, glucose content fructose content and color, while antioxidant activities were determined using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical and ferric reducing/antioxidant power (FRAP) assays, and by measuring the polyphenols contents.

Materials and Methods

Chemicals and reagents

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

Samples

'Hom Thong' banana (*Musa acuminata* 'Gros Michel') fruits were harvested during March 2015.

One-stage and two-stage fermentation

Banana vinegar samples were produced from banana pulp using one-stage and two-stage fermentation. Prior to the fermentation process, 1 kg of banana pulp was added to 1 kg of distilled water and thoroughly blended to prepare banana puree. To avoid undesirable microbial growth, 0.2 g/L potassium metabisulfite was added to the puree and was allowed to settle overnight at room temperature. For one-stage acetic acid fermentation, 1,242 mL of banana puree was added to absolute alcohol (analytical grade) to a final volume of 1,350 mL to provide an alcohol content of 8% and the mixture was transferred to a glass vessel and inoculated with *Acetobacter pasteurianus* TISTR 521 which was grown in glucose yeast broth at a ratio of 10% (volume per volume; v/v). The fermentation was carried out for 20 d at 30ºC on an incubated shaker (150 g). In the two-stage fermentation, 1,800 mL of banana puree was adjusted to 18ºBrix using sucrose syrup and the pH was adjusted to 4.47 using acetic acid. The mixture was then transferred to a glass vessel and inoculated with *Saccharomyces cerevisiae* TISTR 5049 which was grown in yeast malt broth at a ratio of 10% (v/v). Following alcoholic fermentation, the wine was collected using centrifugation at 8,000×*g* at 4ºC for 10 min, filtered through 0.45 µm filter paper and stored at 4ºC when not in use. Acetic acid fermentation was performed using the obtained wine, with the alcohol content adjusted to 8 % (v/v) and the liquid was transferred to a glass vessel and inoculated with *A. pasteurianus* TISTR 521 at a ratio of 10% (v/v). The fermentation was carried out for 20 d under the same conditions. A 100-day aging process was also performed to observe changes in the color of the vinegars.

Sampling was performed at given time points to collect the one-stage and two-stage fermented vinegars using centrifugation at 8,000×*g* at 4ºC for 10 min. The obtained vinegars were filtered through 0.45 µm filter paper and stored at 4ºC in microtubes prior to analysis.

Physicochemical properties

The pH values of the vinegars were measured using a pH meter (Sartorius; Goettingen, Germany) and calibrated using buffer solutions (Mettler Toledo; Greifensee, Switzerland). The total soluble solids values of the vinegars were measured using an AllA France refractometer (AllA France; Chemillé en Anjou, France) calibrated with distilled water. The values were expressed as ºBrix (Akbas and Cabaroglu, 2010).

The analysis of the alcohol, acetic acid, glucose and fructose contents was performed on a Shimadzu HPLC-RID system (Shimadzu, Japan) consisting of Shimadzu LC-20AD pumps and a RID-10A refractive index detector. Samples of wine and vinegar were placed in a microtube closed with parafilm M at 4ºC and kept for 1 mth. The analytical column was an Aminex HPX-87H column (300 mm × 7.8 mm internal diameter 9 µm, Bio-Rad Laboratories Inc.; city, USA) coupled to a cationic exchange precolumn (Bio-Rad Laboratories Inc.; California, USA). H_2SO_4 (5 mM) was used as the mobile phase. The injection volume was 20 µL with a flow rate of 0.6 mL/min. The column temperature was set at 45ºC.

The color of the aged vinegars was measured using a chromameter (Konica Minolta Chromamètre CR-400; Roissy CDG, France) calibrated using a standard calibration scale (El Sheikha et al., 2010). The color parameters for the color change of the vinegars were quantified using the Hunter *L* (whiteness/darkness), *a* (redness/greenness), and *b* (yellowness/blueness) system (McLaren, 1980).

Total phenolics contents

The total phenolics contents of the 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% Na_2CO_3 solution. After 30 min incubation at room temperature, the absorbance was measured at 765 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu; Tokyo, Japan). The results were expressed as micrograms of gallic acid equivalents in 1 mL of sample (μg GAE/mL).

2,2-Diphenyl-1-picrylhydrazyl hydrate radical-scavenging activity

The antioxidant activity of the vinegars was evaluated using DPPH radical assay (Brand-Williams et al., 1995) in which the 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 the incubation period, the absorbance was measured at 517 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu; Tokyo, Japan), and the 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 the control and sample, respectively.

Ferric reducing/antioxidant power assay

The antioxidant activity of the vinegars was also determined using FRAP assay as described by Benzie and Strain (1999) with some modifications. The FRAP reagent was prepared by mixing 10 mM TPTZ solution in 40 mM HCl, 20 mM iron (III) chloride and 300 mM acetate buffer (pH 3.6) at a ratio of 1:1:10 (volume per volume per volume), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37ºC in a water bath prior to use. An amount of 20 μL of the vinegar sample was added to 180 μL of the FRAP reagent, mixed thoroughly and incubated for 30 min. The absorbance was measured at 595 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu; Tokyo, Japan). The results were expressed as micromoles of ferrous ion per milliliter (μM Fe(II)/mL).

Statistical analyses

Statistical analyses were performed using the Statistical Program for Social Sciences software package (SPSS Corporation; Chicago, IL, USA) version 11.0 for Windows. Data for all measurements were obtained in triplicate and were expressed as the mean ± SD. Data were analyzed using one-way analysis of variance and Duncan's multiple range test was used to compare means. The significance threshold was set at .05.

Results and Discussion

Physicochemical properties of banana wine

Table 1 presents the physicochemical properties (pH, total soluble solids (TSS), acetic acid, alcohol, glucose, fructose) of the banana puree, with TSS and pH adjustments used as the substrate for two-stage fermentation for the banana wines obtained during the first step (alcoholic) of the two-stage fermentation. The banana purees used as the substrates for two-stage fermentation had pH values of 4.47. The pH of the wines decreased from 4.20 to 4.07 over the 3-day alcoholic fermentation in the two-stage fermentation process. The initial TSS value for the banana purees used for the two-stage fermentation was 18. During the alcoholic fermentation of the two-stage fermentation process, the TSS values of the wines sharply dropped from 10.07 °Brix to 3.53 °Brix over the 3-day fermentation period, indicating the utilization of sugars for alcohol production. Furthermore, during the alcoholic fermentation of the two-step fermentation process, glucose was rapidly utilized for alcohol production and was depleted at the end of the fermentation, which was in good agreement with Wang et al. (2012a) who utilized a mixed culture of *S. cerevisiae* and *A. pasteurianus* for acetic acid production. In a similar manner, a sharp reduction in the fructose contents was observed during alcoholic fermentation, with a residual content of 1.45% at the end of fermentation. In general, yeasts ferment only carbohydrates with six carbon atoms (hexoses), and glucose and fructose are fermented with the same degree of vigor (Rainieri and Zambonelli, 2009). However, in the present study, glucose was utilized more rapidly than fructose, perhaps because *Saccharomyces cerevisiae* yeast strains preferentially ferment glucose (Solieri et al., 2006).

Table 1 Physicochemical properties (mean±SD) of banana wine.

Time	pH	TSS (ºBrix)	Acetic acid (%v/v)	Alcohol (%v/v)	Glucose (%v/v)	Fructose (%v/v)
Day 0	4.47 ± 0.01^{a}	18.00 ± 0.00^{a}	0.16 ± 0.00^{ns}	1.32 ± 2.25^{d}	10.77 ± 1.45^{a}	6.97 ± 0.41^{a}
Day 1	4.20 ± 0.01^{b}	10.07 ± 0.12^{b}	0.19 ± 0.00^{ns}	5.82 ± 0.03^{c}	4.47 ± 0.11^{b}	5.39 ± 0.01^{b}
Day 2	4.07 ± 0.02^{c}	5.00 ± 0.00^{c}	0.20 ± 0.00^{ns}	10.46 ± 0.05^{b}	0.16 ± 0.04^{c}	3.19 ± 0.00^{c}
Day 3	4.07 ± 0.01^{c}	3.53 ± 0.12^{d}	0.20 ± 0.00^{ns}	12.04 ± 0.02^{a}	0^{c}	1.45 ± 0.01^{d}

v/v = volume per volume; ns = not significant different.

Means with different superscripts in the same column are significantly different ($p < .05$).

Physicochemical properties of the one-stage and two-stage fermented vinegars

Fig. 1 presents the physicochemical properties (pH, TSS, acetic acid, alcohol, glucose, fructose) of the one-stage and two-stage fermented vinegars. The pH of the one-stage and two-stage fermented vinegars dropped from 5.09 to 3.21 and from 4.06 to 3.14, respectively. (Fig. 1A). The TSS of the one-stage and two-stage fermented vinegars ranged from 13.60°Brix to 15.00°Brix and from 4.87°Brix to 4.13°Brix, respectively. (Fig. 1B)

Acetic acid bacteria can convert ethanol to acetate via two consecutive steps using membrane-bound quinoproteins (ethanol dehydrogenase and acetaldehyde dehydrogenase) and the ethanol is oxidized to acetaldehyde by alcohol dehydrogenase and then aldehyde dehydrogenase oxidizes it to acetic acid. *Acetobacter* uses pyrroloquinoline as the preferred hydrogen acceptor that transfers electrons generated from these reactions. Electrons are initially transferred to ubiquinone, which is re-oxidized by a membrane-associated oxidase. Finally, oxygen is the last electron acceptor, resulting in the formation of H_2O and a proton motive force necessary for energy production through a membrane-bound adenosine triphosphatase (ATPase). As such, Acetic acid bacteria are thought to have an absolute requirement for oxygen (Vidra and Nemeth, 2018).

Over the 20-day fermentation process, the level of total acidity (acetic acid) in the one-stage fermentation increased from 0.07% to 6.36% from day 0 to day 10 but gradually decreased during days 15–20 because of the absence of ethanol (Fig. 1C) and due in part to the death of the culture. Moreover, when bacteria use acetic acid as the carbon source, acetate peroxidation may occur, leading to overoxidation and the formation of CO_2 and H_2O (Tesfaye et al., 2000; Hidago et al., 2010). On the other hand, the level of total acidity (acetic acid) in the two-stage fermented vinegars increased from 0.19 to 5.13% (Fig. 1D). These findings were in agreement with Ozturk et al. (2015) which demonstrated that the pH of traditional homemade Turkish vinegars was in the range 2.63–3.90 with total acidity in the range 0.32–5.50%. Many types of vinegar have been assessed for their acidity in several previous studies and the acidity of those vinegars was in the range 2.34−5.61%, including blueberry vinegar (2.34%; Kim et al., 2012), pine vinegar (5.21%; Chen et al., 2011), purple sweet potato *Makgeolli* vinegar (5.61%; Chun et al., 2014) and *Hericium erinaceus* vinegar (4.09%; Li et al., 2014).

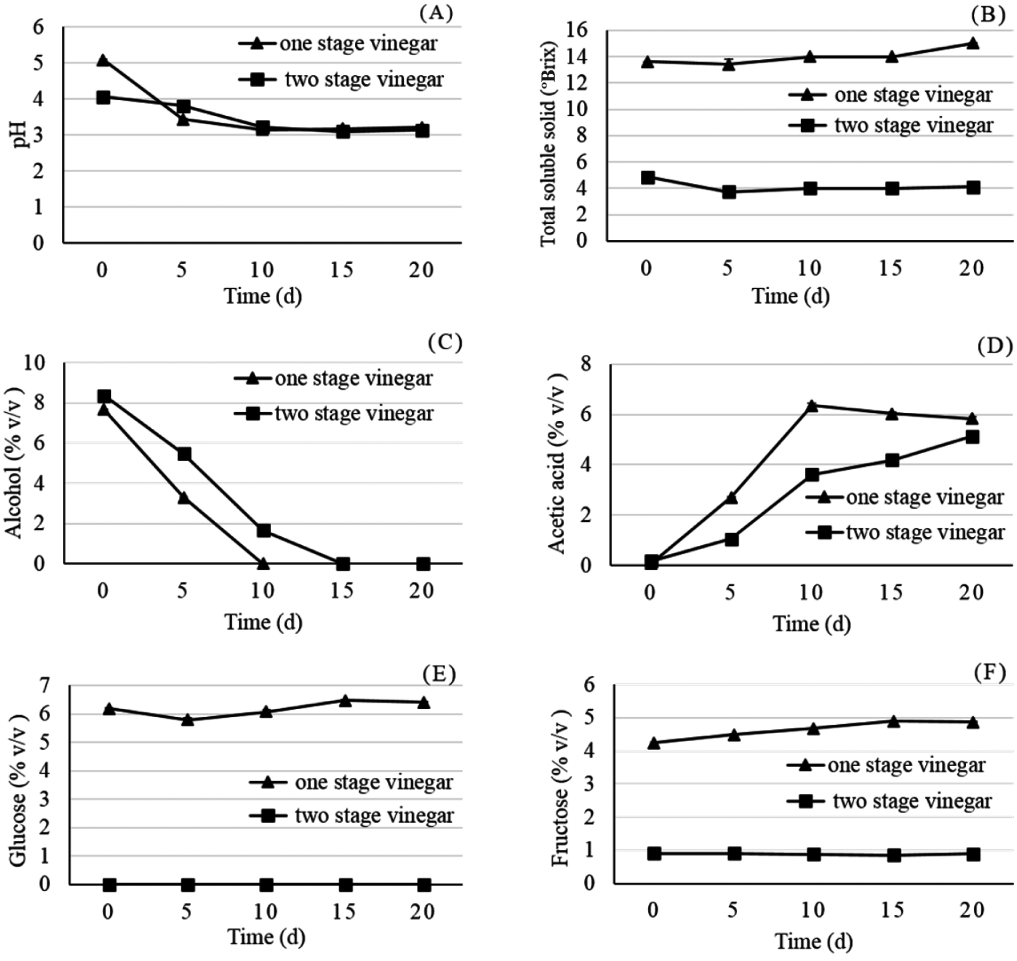

Fig. 1 Physicochemical properties of one-stage and two-stage fermented vinegars during a 20-day fermentation process, (A) pH; (B) total soluble solids; (C) alcohol; (D) acetic acid; (E) glucose; (F) fructose; v/v = volume per volume.

The percentage of acetic acid in the one-stage fermented vinegar was greater than that in the two-stage fermented vinegar, which might have been due in part to the fact that the initial level of glucose in the one-stage fermentation (6.20 %) was higher than that in the two-stage fermentation (0.00 %). Following the 5-day fermentation process, the percentage of glucose in the one-stage fermented vinegar gradually decreased, indicating the utilization of glucose by acetic acid bacteria, which was consistent with Kim et al. (2005) demonstrating that the produced acetic acid concentration increased with increasing glucose or glycerol concentration up to 10 g/L. When 10 g/L of glucose or glycerol was used as a carbon source for acetic acid production during days 10−20 of the one-stage fermentation, the glucose content increased (6.09−6.42%) because *Acetobacter* and *Gluconacetobacter* were able to grow floating in a static culture, producing a pellicle on the surface of the medium. The pellicle is a collection of cells that are tightly associated through the extracellular *polysaccharide* (EPS) on the cell surface. The pellicle polysaccharides are a homopolysaccharide of cellulose. The composition of the EPS of *Acetobacter* species has been studied and consists of glucose, mannose, galactose and glucuronic acid (Perumpuli et al., 2014).

Total phenolic contents and antioxidant activities of the wine

Table 2 shows the changes in the levels of the antioxidant activities and the total phenolic contents of the banana wines. The banana puree with TSS and pH adjustments used as the substrate for the two-stage fermentation showed DPPH radical-scavenging activity, which was expressed as an inhibition of 75.03%. Furthermore, the FRAP value in the banana purees used as the substrate for the two-stage fermentation processes was 741.58 µM/L. Banana puree used as the substrate for banana wine fermentation had a level of total phenolic compounds of 136.14 µg/mL.

Changes in the levels of antioxidant activity and the total phenolic compounds in the banana wines obtained during the first step of the two-stage fermentation were examined over a 3-day fermentation period. It was evident that the levels of antioxidant activity and total phenolic compounds gradually decreased as the process progressed. The % inhibition varied from 82.88% to 65.55% while the FRAP values were in the range 1,386.44–1318.8 µM/L.

A likely explanation for the different behaviors of the FRAP and DPPH assays might be the different reaction mechanism of the substances in the reaction medium. The FRAP assay takes advantages of electron-transfer reactions, herein the ferric salt $Fe(III)(TPTZ)_2Cl_3$ was used as an oxidant. In contrast, DPPH is one of a few stable and commercially available organic nitrogen radicals ((Huang et al., 2005). This method involves a single-electron transfer reaction which measures the antioxidant reducing capacity. The total phenolic contents decreased from 229.10 µg/mL to 172.92 µg/mL (day 1 to day 3), which was consistent with Towantakavanit et al. (2011), elucidating that the decrease in the total phenol levels could have been due to fermentation process condensation and polymerization reactions as well as the formation of oxidative products and precipitation. Variations in the levels of the antioxidant capacity and total phenolic compounds have been reported in a variety of wines. For example, Garaguso and Nardini (2015) reported that the antioxidant activity measured in terms of the FRAP values in conventional wines and organic red wines was in the range 16.4–22.5 mM Fe(II) Eq and 15.4–31.4 mM Fe(II) Eq, respectively. Furthermore, the total phenolic contents detected in conventional wines and organic red wines varied from 3,043 mg/L to 5775 mg/L and from 3007 mg/L to 5653 mg/L, respectively. Moreover, Lucena et al. (2010) studied the total phenolic contents in selected Brazilian wines and found that red wine produced from the Tannat grape variety had the greatest total phenolic content of 5.9 ± 0.5 g GAE/L. (Towantakavanit et al., 2011) examined the total phenolic compounds in wines produced from new cultivars of Korean kiwifruit and observed that among the studied wines, wine made from 'Daehung' had the greatest total phenolic content of 790 mg/L.

Table 2 Antioxidant activity and total phenolic content (mean±SD) of banana wine.

Sampling time	DPPH (%)	FRAP (µM/L)	TPC (µg/mL)
Day 0	75.03 ± 1.11^{b}	741.58 ± 1.420^{c}	136.14 ± 0.12^{d}
Day 1	82.88 ± 0.62^{a}	1386.44 ± 4.87^{a}	229.10 ± 0.39^{a}
Day 2	64.64 ± 0.74^{c}	1369.09 ± 2.69^{a}	197.60 ± 0.44^{b}
Day 3	65.55 ± 0.40^{c}	1318.80 ± 7.97^{b}	172.92 ± 0.73^{c}

DPPH = 2,2-diphenyl-1-picrylhydrazyl hydrate; FRAP = ferric reducing/antioxidant power; TPC = total phenolic content.

Means with different superscripts in the same column are significantly different ($p < .05$).

Total phenolic contents and antioxidant activities of the vinegars after a 20-day fermentation process

During the 20-day fermentation process, variations in the levels of the antioxidant capacity and the total phenolic compounds were identified in the one-stage and two-stage fermented vinegars and the results are given in Fig. 2. The DPPH radical-scavenging activity, which expressed as inhibition, increased from 69.38% to 92.94% from day 0 to day 10 of the one-stage fermentation and dropped to 80.27% on the final day. Similarly, the FRAP values observed for the one-stage fermented vinegars varied from 1086.73 µM/L to 1494.69 µM/L during days 0−10 of the fermentation and decreased to 1274.18 µM/L on the final day. For the two-stage fermentation, the DPPH radical-scavenging activity was in the range 82.62–84.24% during days 0−5 of the fermentation and reduced to 73.20% on the final day. Meanwhile, the FRAP values observed for the two-stage fermented vinegars increased from 1,151.42 µM/L to 1,186.87 µM/L during days 0–10 of the fermentation and fell to 1,001.31 µM/L on the final day of the fermentation. The current results for the antioxidant activity of one and two stage vinegar were greater than for *Hericium erinaceus* vinegar (16.26%; Li et al., 2014) and sweet potato *Makgeolli* vinegar (67.63%; Chun et al., 2014). Furthermore, the total phenolic contents in the one-stage and two-stage fermented vinegars decreased from 178.26 µg/mL to 92.67 µg/mL and from 131.27 µg/mL to 68.01 µg/mL, respectively. The results obtained in this study were in good agreement with Andlauer et al. (2000) which demonstrated that the presence of polymeric polyphenols might explain the high content

of total phenols as polymeric phenols are oxidized more slowly than monomeric phenols because the acetification process diminished the total phenols only by approximately 8%. The antioxidant activity and the total phenolic contents in the one-stage fermented vinegars were greater than in the two-stage fermented vinegar, which was perhaps due to the fact that the oxidization of polyphenols in the two-stage fermented vinegars occurred twice (in the alcoholic and acetification fermentations). The levels of antioxidant activity and total phenolic compounds have been documented to vary across different types of vinegar. For example, Li et al. (2014) determined the antioxidant activity and total phenolic contents of *Hericium erinaceus* vinegar and found that the studied vinegar had a DPPH radical-scavenging activity of 16.26% and a total phenolic content of 7.75 mg GAE/mL. Moreover, Chun et al. (2014) examined the antioxidant capacity and total phenolic contents of sweet potato *Makgeolli* vinegar and this vinegar had an inhibition of 67.63% and a total phenolic content of 327.14 mg GAE/mL. Ozturk et al. (2015) also studied the antioxidant capacity in traditional homemade Turkish vinegars and found that the DPPH radical-scavenging activity was in the range 0.53–90.36%, with the greatest antioxidant level in Pomegranate vinegar. The antioxidant capacities were also examined in commercial vinegar drinks in Korea, and blackberry vinegar had the greatest antioxidant activity of 12.07% (Kim et al., 2012). Moreover, persimmon vinegar and traditional balsamic vinegar of Modena were reported to have total phenolic contents of 324 mg GAE/kg (Ubeda et al., 2011) and 7,646 µg Chloroformic extract /ml (Bertelli et al., 2014), respectively.

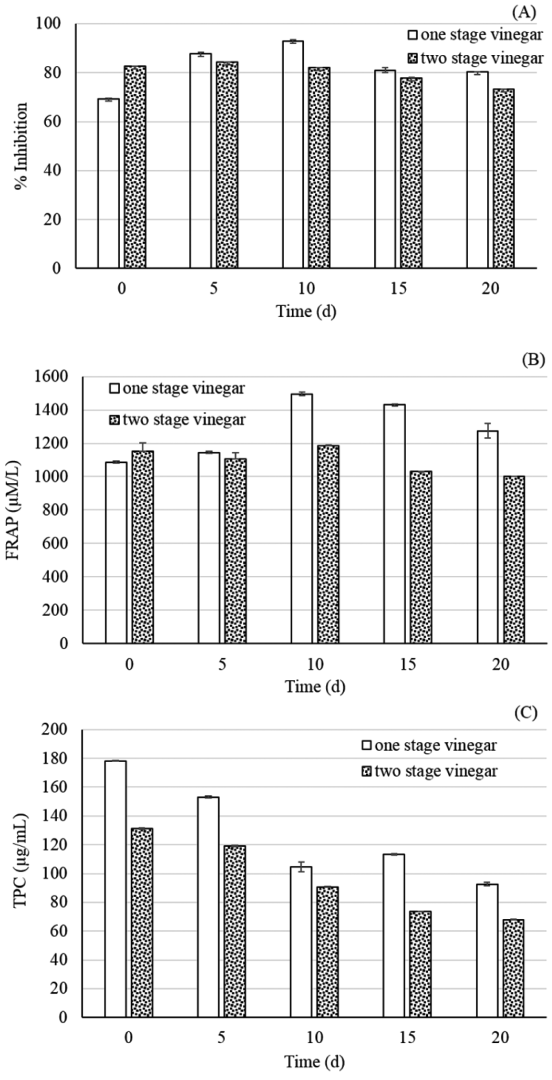

Fig. 2 Antioxidant activities of one-stage and two-stage fermented vinegars during a 20-day fermentation process, (A) 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH); (B) ferric reducing/antioxidant power (FRAP); (C) total phenolic contents (TPC); error bars indicate ± SD.

Color properties of the aged vinegars

After the acetous fermentation process, the one and two stage fermented vinegar were age in glass bottle in ambient temperature. The vinegars were sampled for color measurement every 20 d from days 0–100 day (Fig. 3).

The color parameters for the color change of the vinegars produced using the one-stage and two-stage fermentation were quantified using the Hunter *Lab* system and the results are given in Fig 3. The *L* values observed for the one-stage fermented vinegar decreased from 87.15 to 86.58 while those calculated for the two-step fermented vinegar increased from 87.45 to 88.03 for days 0 to 100, respectively. Because it is a measure of the color in the light-dark axis, decreasing values indicate that the one-stage fermented vinegar became darker with an increase in aging time. The initial and final *a* values for the one-stage and two-stage fermented vinegars varied from -3.82 to -4.13 and from -3.91 to -4.08, respectively, as the aging time increased. The increase in the *a* values indicated that the greenness of the vinegars increased due to the aging process.

Conversely, an increase in the *b* values was observed during the aging process. The initial and final *b* values of the one-stage and two-stage fermented vinegars increased from 2.17 to 3.58 and from 2.34 to 2.50, respectively, suggesting an increase in the yellowness of the vinegars with extended aging time. The overall color of the vinegars changed slightly during the aging period, which was in good agreement with Li et al. (2014). The color parameters quantified for the vinegars in the present study were inconsistent with Ozturk et al. (2015), which reported that the *L* values of industrial and traditional homemade Turkish vinegars varied from 0.28 to 20.15. The changes in the color of the vinegars were primarily due to phenolic compounds involved in different reactions that finally resulted in the formation of the enzymatic browning reaction. These enzymatic reactions result in browning of the product and are activated by the presence of certain concentrations of heavy metals, for example Fe and such reactions are in some way responsible for the marked increase in the absorbance produced in the vinegar during the physicochemical phase (50–75 yr) and contribute to an increase in the color and aroma of the product (Palacios et al., 2002).

Overall, the current study examined and compared the levels of acetic acid and antioxidant activities of the banana vinegars produced though one-stage and two-stage fermentation processes. The results presented in Fig. 1 and Fig. 2 show that the one-stage fermented vinegar appeared to contain remarkably greater levels of acetic acid as well as higher antioxidant activity than the two-stage fermented

vinegar during both fermentation processes. The findings suggested that the one-stage fermentation process is more promising in the production of banana vinegars with high amounts of acetic acid and antioxidants; it should be further developed for vinegar production since it is rapid and cost-effective. Further studies should investigate the effects of the fermentation containers on changes in the levels of acetic acid as well as antioxidants.

Conflict of Interest

The authors declare that there are no conflicts of interest

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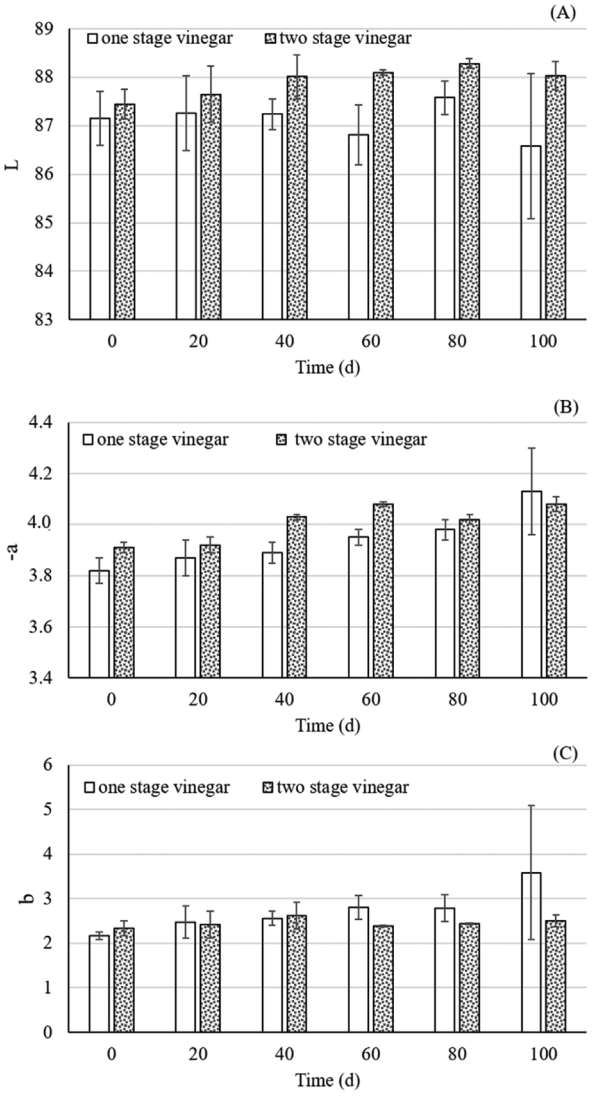

Fig. 3 Color parameters of the one-stage and two-stage fermented vinegars over a 100-day aging process, (A) L; (B) -a; (C) b; error bars indicate ± SD

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