Production of vinegar from purple sweet potato in a liquid fermentation process and investigation of its antioxidant activity

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Abstract *Acetobacter pasteurianus* JST-S was screened from solid fermented grains of vinegar in China, identified by molecular analysis, and used for the production of purple sweet potato vinegar using purple sweet potato as the substrate. By orthogonal experiment, maximum total acid concentration (4.26% [v/v]) was achieved under optimized conditions as follows: fermentation time, 3.5 days; ethanol content, 9% v/v; and inoculum size, 8% v/v. During the production of purple potato vinegar, the anthocyanin concentration decreased from 652.07 to 301.73 µg/mL. The antioxidant activity of products, including diphenyl-picryl hydrazide radical-scavenging capacity (above 60%), reducing power (above 0.47), and hydroxyl radical-scavenging capacity (above 46%), showed positive linear regression ($P < 0.01$), which could be related with the changes in anthocyanin concentration and antioxidant activities at different stages of vinegar fermentation. The acetic acid and other non-phenolic antioxidants in purple sweet potato vinegar may have contributed to the antioxidant activities. Results of these studies may provide a reference for the industrial production of vinegar by liquid fermentation of purple sweet potato.

Keywords *Acetobacter pasteurianus* · Purple sweet potato vinegar · Anthocyanin concentration · Antioxidant activity · Liquid fermentation

Introduction

Vinegar is widely used in food, medical, and other industries. A recent report described the production of vinegar by two-stage liquid fermentation using fruits or vegetables. Acetic acid and other organic acids are the main components of fruit vinegar. Acetic acid is important for the complete transformation of sugars and fats into energy and may be effectively used to avoid excessive accumulation of these energy materials in the body. In addition, acetic acid may promote softening of the blood vessels and control blood cholesterol level. Organic acids are used to maintain pH balance and stability in vivo for normal metabolic and physiologic functions (Nazioglu et al. 2014). Fruit vinegar contains various kinds of nutritional components derived from fruits and vegetables. There is a rising demand for fruit vinegar in the market all over the world.

Purple sweet potato is not only rich in nutrients from the ordinary sweet potato, but also contains selenium, anthocyanin, flavonoids, glycoprotein, pectin, chromogenic acid, and other functional components. As a result, purple sweet potato displays multifaceted actions, including scavenging of free radicals, antioxidative activity, prevention and treatment of cardiovascular diseases, and other physiological functions (Harakotr et al. 2014; Sun et al. 2015; Xu et al. 2015; Cai et al. 2016; Zhang et al. 2016). In recent years, the production of purple sweet potato has increased and most of it is consumed as a fresh food. At present, a few kinds of products are manufactured from purple sweet potato, such as purple potato powder, freeze-dried food,

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and purple potato beverages (Diaz et al. 2014; Fukui et al. 2015).

It is important to improve the utilization rate of purple sweet potato. Producing vinegar using purple sweet potato in a high-efficiency liquid fermentation process is a good way to improve vinegar manufacturing to meet the current market demand. Fukui et al. (2015) provided a new high concentration brewing method for the production of purple sweet potato vinegar with a deep color and high functionality and studied its functions using animal experiments. Chun et al. (2014) investigated different contents of rice *makgeolli* and purple sweet potato *makgeolli* and performed a detailed comparison of the quality characteristics of vinegar produced using rice and purple sweet potato *makgeolli* during storage. However, further systematical studies on the fermentation process and the relation between antioxidant activities and composition changes are needed. Here, we screened and isolated a strain of acetic acid bacteria (AAB) and used it in the production process of purple sweet potato vinegar by an orthogonal experiment. The changes in the anthocyanin concentration and antioxidative activities of the products at different stages of acetic acid fermentation were investigated.

Materials and methods

Microorganism and media

In this study, purple sweet potato was obtained from the Zhougudui Market of Hefei, Hefei, Anhui Province, China. Dry yeast powder was purchased from Angel Yeast Co. Ltd., Yichang, Hubei Province, China. Both α -amylases and glucoamylase were provided by Jiangsu Ruiyang Biotech Co., Ltd.

The solid culture medium [0.5 g yeast extract, 2 g glucose, 0.3 of KH_2PO_4 , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 2 g agar per 100 mL potato juice (200 g/L)] was sterilized at 1×10^5 Pa for 20 min, and 2% (v/v) ethanol was added into it before making the plates. The separate medium (1 g glucose, 1 g yeast extract, 2 g agar, and 1 g CaCO_3 per 100 mL distilled water, pH 4.5) was sterilized at 1×10^5 Pa for 20 min, and 3% (v/v) ethanol was added into it before making a flat plate. In addition, the preculture medium (1.1 g glucose, 1.1 g yeast extract, 0.33 g KH_2PO_4 , and 0.11 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per 100 mL distilled water) was sterilized at 1×10^5 Pa for 20 min, and 2% (v/v) ethanol was added into it before inoculating.

Screening and isolation of AAB

For screening of AAB, 2 g solid fermented grains of vinegar (Di Boshi Brewing Machine Co., Ltd., Yantai,

China) were cultured in 250 mL of flask containing 50 mL preculture medium at 150 rpm for 24 h at 32 °C. Following incubation, 10 mL of culture broth was transferred into 50 mL of preculture medium with 4% (v/v) acetic acid and 6% (v/v) ethanol and cultured at same conditions. This step was repeated for 6 days to obtain the culture of AAB, which was subjected to preliminary screening. The culture liquid was statically placed in an incubator (Yi Heng Scientific Instrument Co., Ltd., Shanghai, China) at 32 °C for 3 days to obtain the white film. The white film was carefully picked using an inoculation loop and developed on a separate media at 32 °C for 48 h to obtain single colonies with larger transparent circles. Ten isolates of strains were cultured on solid culture medium for preserving the screened strains and numbered from 1 to 10.

Identification of AAB

After identification of physicochemical characteristics as per a previous report (Cowan 1934), the screened bacteria were grown in preculture medium at 150 rpm for 3 days at 32 °C. Genomic extraction of DNA from the bacteria was performed as described by Sambrook et al. (1982). Specific primer sequences for the polymerase chain reaction (PCR) amplification of 16S rDNA, 27F (5'AGAGTTTGTATCTTGGCTCAG-3') and 1942R (5'TACGGCTACCTTGTTACGACTT-3') were designed (Brosius et al. 1980; Weisburg et al. 1991). The PCR products were purified using a PCR purification kit (TaKaRa Biotechnology Co., Ltd., Dalian, China) as per the manufacturer's instructions and sequenced (Sangon Biotech Co., Ltd., Shanghai, China). Sequencing results were stitched using DNASTAR software to obtain the amplified 16S rDNA nucleotide sequences, which were submitted to GenBank (NCBI) (<http://blast.ncbi.nlm.nih.gov/>), and compared with the 16S rDNA sequences of the strains in these databases using Basic Local Alignment Search Tool (BLAST).

The process of production of purple sweet potato vinegar

The process of production of purple sweet potato vinegar is shown in Fig. 1. The details are as follows.

Preparation of purple sweet potato pulp

Fresh purple sweet potato were selected, cleaned, and cooked in presence of vapor for 40 min. Then, 100 g of cooked purple sweet potato was mashed in 1000 mL water. The pH value of the mashed purple sweet potato was adjusted to 7.5 with sodium hydroxide (NaOH) or citric acid and 70 μL of high-temperature α -amylase was added per 100 g mashed purple sweet potato. The mixture was

Fig. 1 The process of production of purple sweet potato vinegar. 1, fresh purple sweet potato pulp, 1000 g of fresh purple sweet potato was mashed with 500 g water; 2, mashed purple sweet potato pulp; 3, purple sweet potato liquefied pulp; 4, purple sweet potato saccharified pulp; 5, purple sweet potato wine; 6, purple sweet potato vinegar

liquefied at 85 °C for 1.5 h, and the pH of the liquefied pulp was adjusted to 5.0 with citric acid, followed by the addition of 7.7 mL glucoamylase per 100 g liquefied pulp. The pulp was saccharified at 55 °C for 2.5 h to obtain a purple sweet potato saccharifying pulp (Xu et al. 2015; Zhang et al. 2016).

Alcohol fermentation

We inoculated 20 g yeast into a 250-mL flask containing 100 mL sterile water and cultured it at 30 °C for 30 min as a yeast seed (Diaz et al. 2014).

Glucose was added to the purple sweet potato saccharifying pulp at a final sugar concentration of 10% (w/v) and 50 mL purple sweet potato saccharifying pulp containing 10% (w/v) glucose was poured into 250-mL flask. The pulp was stirred at 105 °C for 5 min and treated with 2 mL yeast seed for about 4 days at 28 °C to obtain a purple sweet potato wine.

Acetic fermentation

The isolated strain was first cultured on a solid culture medium at 30 °C for 48 h, followed by its inoculation into a 250-mL flask containing 50 mL sterilized preculture medium. The strain was cultured in a rotary shaker at a speed of 150 rpm at 30 °C for 24 h as a seed liquid.

We used 1 mL seed liquid to inoculate 50 mL sterilized (105 °C for 5 min) purple sweet potato wine with 9% (v/v) ethanol. The culture was incubated under same conditions for 4 days to obtain purple sweet potato vinegar. Following fermentation, the purple sweet potato vinegar was sterilized

using pasteurization (Chun et al. 2014; Fukui et al. 2015) as the final purple sweet potato vinegar.

Sample treatment

All original samples were centrifuged (1000 rpm) at 5 °C for 10 min and the supernatant used as a sample for analysis.

Analysis of ethanol

Briefly, 1 mL purple sweet potato wine supernatant was diluted with 9 mL methanol and filtered through a 0.22-μm membrane. The modified method of gas chromatography (Song et al. 2015) was used to determine the concentration of ethanol by direct injection into an Agilent 7890A gas chromatograph (Agilent) equipped with a split/splitless injector, electronic flow control (EFC), and a flame ionization detector (FID). An aliquot of 2 μL of this mixture was injected (split 1:30) into a Agilent J and W GC Columns DB-624 fused silica capillary column of 25 m × 0.2 mm and 1.12 μm film thickness (Agilent, The USA). Instrumental conditions were as follows: injector temperature of 150 °C, detector temperature of 200 °C, nitrogen as carrier gas at 3.3 mL/min, and nitrogen as make-up gas at 30 mL/min. Flow rates of detector gas hydrogen and air were 40 and 400 mL/min, respectively. The temperature program was as follows: 50 °C for 5 min, increased to 200 °C at a rate of 4 °C/min and held at 200 °C for 5 min. Total running time was 15 min.

Analysis of total acid concentration

Acetic acid concentration was measured by the acid–base titration method (Chun et al. 2014; Qi et al. 2014; Diba et al. 2015; Xia et al. 2015). Briefly, 1 mL sample was added into 50 mL distilled water in a 150-mL beaker and titrated with 0.1 mol/L NaOH using phenolphthalein as the indicator until the color of the mixture was pink for 30 s. The consumption of 0.1 mol/L NaOH was V_1 . Distilled water was used as control and the consumption of 0.1 mol/L NaOH in presence of distilled water was V_2 . AAC was calculated as below:

$$\text{AAC (\%)} = (V_1 - V_2) \times C \times 60, \quad (1)$$

where AAC = acetic acid concentration in the sample (%) and C = concentration of standard NaOH solution (mol/L).

Measurement of colony-forming units (CFU)

We determined CFU using the flat colony counting method. Briefly, 1 mL of purple sweet potato vinegar was

poured into the solid culture medium and cultured at 30 °C for 48 h. The control study was performed using sterile water as blank, and total number of bacterial colony was counted. The original fermentation purple sweet potato vinegar was diluted using sterile water if the total number of bacterial colony was not in the range of 30–300.

Measurement of the total polyphenol content

The total polyphenol content was measured by the Folin–Ciocalteu method (Budak and Guzel-Seydim 2010; Hu and Xu 2011; Chun et al. 2014; Harakotr et al. 2014). Briefly, 5 mg anthocyanidin was dissolved in 10 mL methanol to obtain a 0.5 mg/mL anthocyanidin standard solution. 10.0 g of ammonium ferric sulfate was dissolved by 2 mol/L HCl to 100 mL as the 10% ammonium ferric sulfate, then the mixed ratio of *n*-butyl ethanol, hydrochloric acid and 10% ammonium ferric sulfate was 83:6:1 (v/v) as the mixture. 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mL of the 0.5 mg/mL anthocyanidin standard solution was dissolved to 1.0 mL using methanol. After mixing with 9.0 mL of the mixture, the solutions were heated in boiled water for 40 min, cooled in ice water for 4–5 min, and warmed for about 15 min until the temperature reached about 25 °C. The absorbance of the solutions was measured at 520 nm wavelength with a spectrophotometer (T6, Persee, China) using 1.0 mL methanol and 9.0 mL mixture as a blank.

The regression equation of absorbance (Y) and anthocyanidins concentration (X , $\mu\text{g/mL}$) was analyzed as below:

$$Y = 0.0032X - 0.073. \quad (2)$$

The correlation coefficient $R^2 = 0.9996$ and the linear range was 50–300 $\mu\text{g/mL}$.

Anthocyanidins concentration in the sample was measured using the same method by replacing the 0.5 mg/mL anthocyanidin standard solution. In order to be in a linear range, the sample was diluted with distilled water when necessary and the procyanidin concentration calculated using Eq. 2.

Assay of 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

We measured DPPH scavenging activity as previously described (Terahara et al. 2003; Xu et al. 2007; Sakanaka and Ishihara 2008; Budak and Guzel-Seydim 2010; Chun et al. 2014) with a few modifications. Briefly, 2×10^{-4} mol/L DPPH solution was prepared using 100% ethanol and 2 mL of DPPH solution was added to 2 mL sample. The mixture was well shaken and incubated for 30 min in the dark at 25 °C. The sample was diluted using distilled water when necessary. The absorbance of the

solution was measured at 517 nm wavelength using spectrophotometer. The DPPH scavenging activity (%) was calculated as below:

$$\text{DPPH scavenging activity (\%)} = (1 - [A_1 - A_2]/A_0) \times 100. \quad (3)$$

A_1 = absorbance of mixture containing 2 mL sample and 2 mL DPPH solution, with a mixture of 2 mL 100% ethanol and 2 mL distilled water used as blank. A_2 = absorbance of mixture containing 2 mL sample and 2 mL distilled water, with 4 mL distilled water used as blank. A_0 = absorbance of mixture containing 2 mL DPPH solution and 2 mL distilled water, with a mixture of 2 mL 100% ethanol and 2 mL distilled water used as blank.

Assay of reducing power

The reducing power assay was performed as described by Xu et al. (2007) in phosphate buffer (0.2 mol/L, pH 6.6). Briefly, 1% potassium ferricyanide, 10% trichloroacetic acid (TCA), and 0.1% ferric chloride solution were prepared using distilled water. The sample (1 mL) was diluted using 9 mL phosphate buffer (0.2 mol/L, pH 6.6) and 1 mL of the mixture was mixed with 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was warmed for 20 min at 50 °C and treated with 1 mL 10% TCA, followed by centrifugation at 3000 r/min at room temperature. The supernatant (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL 0.1% ferric chloride. The absorbance of the solution was measured at 700 nm with spectrophotometer. The higher absorbance of the reaction mixture corresponded with a higher reducing power (Verzelloni et al. 2007).

Hydroxyl radical-scavenging ability

The hydroxyl radical-scavenging ability of samples was measured using the salicylic acid method (Xu et al. 2007; Sakanaka and Ishihara 2008; Du et al. 2013). Briefly, 4 mM iron sulfate (FeSO_4) and 4 mM hydrogen peroxide (H_2O_2) were prepared using distilled water and 4 mM salicylic acid was prepared using 100% ethanol. Samples (2 mL) were mixed with 2 mL FeSO_4 solution and 2 mL salicylic acid solution and 2 mL H_2O_2 solution was added to the mixture. The mixture was well shaken and warmed at 37 °C for 30 min. The mixture was centrifuged at 3000 r/min at room temperature if precipitation was observed and the absorbance of the supernatant measured at 510 nm using a spectrophotometer (DU720). The hydroxyl radical-scavenging rate (r) was calculated as below:

$$\text{Hydroxyl radical-scavenging rate (\%)} = (1 - [A_1 - A_2]/A_0) \times 100. \quad (4)$$

A_1 = absorbance of mixture containing 2 mL sample, 2 mL FeSO₄ solution, 2 mL salicylic acid solution, and 2 mL H₂O₂ solution, with 8 mL distilled water as blank. A_2 = absorbance of mixture containing 2 mL sample, 2 mL FeSO₄ solution, 2 mL salicylic acid solution, and 2 mL distilled water, with 8 mL distilled water as blank. A_0 = absorbance of mixture containing 2 mL distilled water, 2 mL FeSO₄ solution, 2 mL salicylic acid solution, and 2 mL distilled water, with a mixture of 2 mL 100% ethanol and 2 mL distilled water used as blank.

Data analysis

Results of experiments are expressed as mean $\pm$ standard deviation ($n = 3$). Orthogonal experiments were performed with repeated trials with a statistical significance ($P < 0.05$) using Microsoft Office Excel (version 2007) statistical analysis program. One-way analysis of variance (ANOVA) was performed with Duncan test (compare all pairs of column) in multiple comparisons and Fisher's protected multiple comparisons was used (Wang 2006; Wu et al. 2011).

Results and discussion

Identification of AAB

The white biofilm of AAB (Fig. 2) was coated on separate media. Table 1 shows the physicochemical characteristics of the strains. Strain 3 capable of using ethanol and glycerol as substrates was a Gram-negative bacterium and

Fig. 2 The velum formed on the surface of culture medium

produced acid. It was identified as *Acetobacter pasteurianus* based on its ability to form biofilm and other characteristics (Cowan 1934; Yamada et al. 1999).

The 16S rDNA nucleotide sequences of strain 3 was submitted to NCBI database (the accession number, MF457917). The strain was found to be most related to *A. pasteurianus* (97% similarity value) (data not shown). It was named as *A. pasteurianus* JST-S, as per the results of colony morphology, physicochemical characteristics, and comparison of homology of 16S rDNA sequence and stored in the lab of the School of Food Science and Engineering, the Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology.

Optimization of acetic acid fermentation conditions by orthogonal design experiment

The purple sweet potato wine contained a low initial ethanol concentration (6%, w/v); therefore, ethanol was added to obtain an appropriate level of ethanol necessary for the production of the desired purple sweet potato vinegar.

According to the results of a single factor variance analysis, fermentation time, initial ethanol concentration, and AAB inoculum had a significant effect ($P < 0.05$) on AAC (data not shown). Therefore fermentation time, ethanol concentration, and inoculum of AAB were optimized by L₉ (3⁴) orthogonal experiment (Table 2). The variance analysis results are shown in Table 3.

From the direct analysis of results described in Table 2, the optimum condition for the production of vinegar was A₂B₂C₃, i.e., the optimum fermentation time was 3.5 days, ethanol concentration was 9% (v/v), and AAB inoculum was 10% (v/v). AAC at the end of the fermentation reached 4.11% (w/v) and corresponded to the fifth test in Table 2. However, computational analysis made it easy to find the optimal combination of A₂B₂C₂ from the values of K_1 , K_2 , and K_3 (the sum of results with each level) of each factor in Table 2. Thus, highest AAC was achieved with a fermentation time of 3.5 days, 9% (v/v) ethanol concentration, and 8% (v/v) AAB inoculum.

To improve the accuracy of data analysis (Wang 2006; Pan and Chen 2008; Wu et al. 2011), null columns and repeated trials were used to estimate the experimental error. Primary and secondary factors were A > B > C, based on the values of MS_A (mean of sum of the square for the factor of time, A), MS_B (mean of the sum of square for the factor of alcohol, B), and MS_C (mean of the sum of square for the factor of inoculum, C), which were 0.081, 0.013, and 0.006, respectively.

Table 3 shows that fermentation time was a highly significant factor that influenced AAC at 99% confidence

Table 1 Identification results of physicochemical characteristics for acetic acid bacteria

Strain	Starch hydrolysis test	Ethanol utilization test	Glycerol utilization test	Gram staining test	Acid production test
1	–	–	+	+	–
2	–	–	+	–	+
3	–	+	+	–	+
4	–	–	–	+	–
5	+	+	–	–	–
6	+	–	–	–	–
7	+	–	–	–	–
8	–	–	–	+	–
9	+	–	–	+	–
10	–	–	–	+	–
<i>Ap. CICC 20001</i>	–	+	+	–	+

Identification of physicochemical characteristics for acetic acid bacteria screened was performed using starch hydrolysis test, ethanol utilization test, glycerol utilization test, Gram staining test, qualitative acid production test. Apart from Gram staining test and qualitative acid production test, a plus sign represents that the strain can use the substrate for the growth, while a minus sign represents the opposite meaning. In Gram staining test, the plus sign represents Gram-positive bacteria and the minus sign represents Gram-negative bacteria. In qualitative acid production test, the plus sign represents the strain can produce acetic acid and the minus sign represents contrary meaning

Table 2 Orthogonal array design and experimental results of the acetic fermentation

Num	Factors				AAC, %	
	Time (days)	EC (%)	Inoculum (%)	e_1		
1	1 (3)	1 (8)	1 (6)	1	3.75	3.78
2	1	2 (9)	2 (8)	2	3.90	3.98
3	1	3 (10)	3 (10)	3	3.86	3.80
4	2 (3.5)	1	2	3	4.10	4.06
5	2	2	3	1	4.10	4.13
6	2	3	1	2	4.02	4.04
7	3 (4)	1	3	2	3.96	4.01
8	3	2	1	3	4.01	4.02
9	3	3	2	1	3.89	4.04
K_1	23.07	23.66	23.62	23.69		
K_2	24.45	24.14	23.97	23.91		
K_3	23.93	23.65	23.86	23.85		
SS_j	0.162	0.026	0.011	0.004		

level, with F-ratios of 46. Ethanol concentration influenced AAC at 95% confidence level, with F -ratios of 6.5. Thus, fermentation time and ethanol concentration had significant effects ($P < 0.05$) on AAC, while AAB inoculums showed no significant effect ($P > 0.05$) on AAC.

The results of the verification tests with optimum conditions of direct and computational analyses (Table 4) suggest that AAC achieved was 4.26% (v/v) with the combination $A_2B_2C_2$. Therefore, the optimal process conditions for purple sweet potato vinegar production were as

follows: fermentation time, 3.5 days; ethanol concentration, 9% (v/v); and AAB inoculum, 8% (v/v).

Acetic fermentation of purple sweet potato vinegar

Purple sweet potato vinegar was produced at optimal values by liquid fermentation (Fig. 3). AAC reached 4.2% in 3.5 days, similar to the acetic acid level produced in the YPG culture medium (3.8–4.2% range). Furthermore, Sakurai et al. (2012) reported that *A. aceti* NBRC14818

Table 3 Variance analysis of the acetic fermentation

Source of variance	SS ^a	F ^b	MS ^c	F ^d	Significant
Time	0.162	2	0.081	46	** ^e
EC	0.026	2	0.013	6.5	* ^f
Inoculum	0.011	2	0.006	3	
e ₁ ^g	0.004	2			
e ₂ ^h	0.019	9			
E ⁱ	0.023	11	0.002		
Sum total	0.222	17			

^a Sum of square for each factor^b Freedom^c Mean of sum of square for each factor^d F-ratios^e $P < 0.01$ ^f $P < 0.05$ ^g Error 1, accumulated from the null columns^h Error 2, accumulated from repeated trialsⁱ Total of errors**Table 4** The plan and results of verification test of the acetic fermentation

Conditions	A ₂ B ₂ C ₃	A ₂ B ₂ C ₂
AAC, %	4.095 ± 0.03 ^a	4.26 ± 0.02 ^b

A₂B₂C₃: fermentation time was 3.5 days, EC was 9% (v/v), and inoculum of AAB was 10% (v/v); A₂B₂C₂: fermentation time was 3.5 days, EC was 9% (v/v), and the inoculum of AAB was 8% (v/v)

^{a,b} The bars in the same assay with different letters show significant differences in each row ($P < 0.05$)

displayed a diauxic growth profile with the addition of ethanol. While acetic acid accumulated during the whole fermentation process, ethanol consumption was observed

only in the first exponential phase. During the second exponential phase, *A. aceti* would consume acetic acid to maintain normal metabolism and cell growth when ethanol was consumed thoroughly, and hence, unavailable for metabolism. The first and second exponential phases were considered as ethanol oxidation and acetate oxidation phase, respectively, but there was no stationary growth phase observed in this study. The biomass accumulation reached its maximum in 2 days and no diauxic growth profile was observed with the accumulation of acetic acid in the medium. On the contrary, a slight decline in the biomass was observed after 2 days. Ethanol was gradually consumed with the accumulation of acetic acid and the ethanol concentration in the medium was less than 0.5% (v/v) until the end of the fermentation.

Analysis of anthocyanin concentration and antioxidant activity

The anthocyanin concentration decreased during the production of purple potato vinegar; it was reported to be 652.07 and 301.73 µg/mL in fresh purple sweet potato pulp and purple potato vinegar, respectively (Table 5), indicative of a decrease of 46.06%. Several factors contributed to the decline in the anthocyanin concentration. Anthocyanins were hydrolyzed by microbial enzymes, resulting in the loss of color (Zhang et al. 2001). At the same time, a long processing time during saccharification at high temperature may have contributed to the thermal degradation of anthocyanins (Kirca and Cemeroglu 2003). The high temperature (105 °C) during fermentation and sterilization may have resulted in a significant difference ($P < 0.05$)

Fig. 3 Acetic fermentation of purple sweet potato vinegar. Filled diamond AAC (acetic acid concentration), filled triangle CFU (colony-forming units), filled square EC (ethanol concentration)

Table 5 Changes of anthocyanins concentration and antioxidant activity in different stages of the acetic acid fermentation

	Anthocyanins concentration ($\mu\text{g}/\text{mL}$)	DPPH radical-scavenging capacity ¹⁰ (%)	Reducing power ¹¹ (OD)	Hydroxyl radical-scavenging capacity ¹² (%)
1	652.07 $\pm$ 13.36 ^a	90.01 $\pm$ 3.07 ^a	0.88 $\pm$ 0.03 ^a	57.66 $\pm$ 1.53 ^a
2	622.40 $\pm$ 10.66 ^b	88.57 $\pm$ 2.39 ^a	0.87 $\pm$ 0.03 ^a	56.45 $\pm$ 2.78 ^{a, b}
3	560.19 $\pm$ 10.67 ^c	78.22 $\pm$ 3.03 ^b	0.80 $\pm$ 0.03 ^b	53.08 $\pm$ 2.81 ^b
4	491.92 $\pm$ 12.76 ^d	75.61 $\pm$ 4.17 ^b	0.72 $\pm$ 0.03 ^c	52.03 $\pm$ 2.17 ^b
5	474.44 $\pm$ 7.14 ^d	69.18 $\pm$ 1.99 ^c	0.63 $\pm$ 0.02 ^d	51.06 $\pm$ 2.58 ^{bc}
6	420.63 $\pm$ 10.76 ^e	63.38 $\pm$ 3.30 ^d	0.58 $\pm$ 0.07 ^e	46.28 $\pm$ 2.03 ^c
7	387.29 $\pm$ 6.23 ^f	60.85 $\pm$ 2.60 ^d	0.51 $\pm$ 0.04 ^f	45.27 $\pm$ 1.85 ^c
8	326.20 $\pm$ 11.70 ^g	69.14 $\pm$ 3.48 ^c	0.51 $\pm$ 0.03 ^f	47.02 $\pm$ 1.58 ^c
9	301.73 $\pm$ 8.87 ^h	67.69 $\pm$ 3.04 ^{c, d}	0.47 $\pm$ 0.03 ^f	46.20 $\pm$ 2.69 ^c

1, fresh purple sweet potato pulp, 1000 g of fresh purple sweet potato was mashed with 500 g water; 2, mashed purple sweet potato pulp; 3, purple sweet potato liquefied pulp; 4, purple sweet potato saccharified pulp; 5, sterile purple sweet potato saccharified pulp; 6, purple sweet potato wine; 7, sterile purple sweet potato wine; 8, purple sweet potato vinegar; 9, sterile purple sweet potato vinegar

¹⁰ DPPH radical-scavenging capacity was determined when samples were diluted by 400 times using distilled water, about 0.35 mg/ml

¹¹ Reducing power was determined when samples were diluted by 4000 times using distilled water, about 4 $\mu\text{g}/\text{ml}$

¹² Hydroxyl radical-scavenging capacity was determined when samples were diluted by 500 times using distilled water, about 0.25 mg/ml; a, b, c, d, e, f, g, h, the bars in the same assay with different letters show significant differences in each column ($P < 0.05$)

(Table 5). No significant differences ($P < 0.05$) were observed in anthocyanin concentrations of purple sweet potato saccharified pulp and sterile purple sweet potato saccharified pulp. During ethanol fermentation, ethanol accumulation may promote polymerization of anthocyanins as well as the reaction of anthocyanins with flavonoids—another reason for color loss (Zhang et al. 2001; Morata et al. 2006). However, studies have shown that the glycosidase metabolism of yeast, AAB, and other microorganisms may lead to anthocyanin degradation (Kirca and Cemeroglu 2003; Morata et al. 2006; Fan et al. 2008; Pilar et al. 2013).

We observed that DPPH radical-scavenging capacity decreased from 90.01% in fresh purple sweet potato pulp to 60.85% in sterile purple sweet potato wine during the production of purple sweet potato vinegar (Table 5). A significant difference was observed in DPPH radical-scavenging capacity ($P < 0.05$) between sterile purple sweet potato wine and purple sweet potato vinegar. The difference may be attributed to the production of acetic acid during acetic fermentation. It was reported that more than 80.7% of the anthocyanins extracted from purple sweet potato were acylated anthocyanins, which were more stable at lower pH than at high pH values (Fan et al. 2008). Pretorius (2000) indicated that anthocyanins may combine with acetates as one of the metabolites and form pyroanthocyanins, which are more stable than anthocyanins. These results showed that the increase in acetic acid during fermentation induced higher concentration of anthocyanins. Studies have confirmed that anthocyanins from

purple sweet potato showed stronger DPPH scavenging activity (Kano et al. 2005). In addition, acetic acid itself has antioxidant activity. All the products showed DPPH radical-scavenging capacity above 60% (Table 5).

Linear regression was analyzed (Table 6) to investigate the contribution of anthocyanin concentration in the antioxidant activity of the products at different stages of vinegar fermentation (Davalos et al. 2005). The linear correlation between anthocyanin concentration and DPPH radical-scavenging capacity was 0.86, indicative of a good positive linear regression with a great significant correlation ($P < 0.01$); however, significant differences ($P < 0.05$) were observed between the decrease in anthocyanin concentration at different stages of vinegar fermentation. As shown in Table 5, DPPH radical-scavenging capacity of these products was markedly higher than 60%, indicative of their positive antioxidant activities.

The reducing power of different products during the production of purple potato vinegar was investigated. The higher OD value corresponded to higher reduction ability (Xu et al. 2007). The reducing power declined from 0.88 in fresh purple sweet potato pulp to 0.47 in sterile purple sweet potato vinegar during the production of vinegar (Table 5). It is interesting that the reducing power failed to increase during ethanol and acetic fermentation and showed no significant differences ($P > 0.05$). The reducing power of different products was above 0.47 (Table 5).

The linear correlation between anthocyanin concentration and reducing power was 0.98, indicative of a good positive linear regression with great significant effects

Table 6 Linear regression of the antioxidant activity vs anthocyanins concentration

R^2	Anthocyanins concentration ($\mu\text{g/mL}$)	DPPH radical-scavenging capacity ^a (%)	Reducing power ^b (OD)	Hydroxyl radical-scavenging capacity ^c (%)
Anthocyanins concentration ($\mu\text{g/mL}$)	1			
DPPH radical-scavenging capacity (%)	0.86** ^d	1		
Reducing power (OD)	0.98**	0.92**	1	
Hydroxyl radical-scavenging capacity (%)	0.94**	0.96**	0.97**	1

^a DPPH radical-scavenging capacity was determined when samples were diluted by 400 times using distilled water, about 0.35 mg/mL

^b Reducing power was determined when samples were diluted by 4000 times using distilled water, about 4 $\mu\text{g/mL}$

^c Hydroxyl radical-scavenging capacity was determined when samples were diluted by 500 times using distilled water, about 0.25 mg/mL

^d The bars show great significant correlation ($P < 0.01$)

($P < 0.01$). No significant differences ($P > 0.05$) were reported for the reducing power of sterile purple sweet potato wine, purple sweet potato vinegar, and sterile purple sweet potato vinegar. The reducing powers of the products were significantly affected by ethanol fermentation ($P < 0.05$), but not acetic acid fermentation ($P > 0.05$) (Table 5). This effect was same during mashing, liquefaction, and saccharification of the purple sweet potato pulp.

Hydroxyl radical is identified as toxic to the body, as it acts as a powerful oxidizing agent and may join nucleotides in DNA and cause strand breakage (Xu et al. 2007). The hydroxyl radical-scavenging capacity of different products at different stages of vinegar fermentation was investigated. There was no significant difference ($P > 0.05$) in the hydroxyl radical-scavenging capacity of sterile purple sweet potato saccharified pulp, purple sweet potato wine, sterile purple sweet potato wine, purple sweet potato vinegar, and sterile purple sweet potato vinegar. Thus, ethanol and acetic fermentation had no significant effects on the hydroxyl radical-scavenging capacity of products. As shown in Table 5, the hydroxyl radical-scavenging capacity of these products was in the range of 46–58%, indicative of their positive antioxidant activities.

During the past 20 years, the relation between anthocyanins concentration and antioxidant activity had been reported (Tsuda et al. 1994; Kano et al. 2005; Jiao et al. 2012). Anthocyanins have been shown to display positive effects on the antioxidant activity. The linear correlation between anthocyanin concentration and antioxidant activities (DPPH radical-scavenging capacity, reducing power, and hydroxyl radical-scavenging capacity) was 0.80, indicative of the good positive linear regression ($P < 0.01$). The antioxidant activities showed positive regression with each other (Table 6), with a linear correlation above 0.90 ($P < 0.01$). Anthocyanin concentration decreased following ethanol and acetic fermentation (Table 5), but no

significant difference was observed in the hydroxyl radical-scavenging capacity of vinegar and wine. These observations indicate the presence of new compounds with higher antioxidant activity in the purple sweet potato wine and vinegar. Vinegar acts as an antioxidant and other antioxidant components in purple sweet potato pulp, such as vitamins, may have improved its antioxidant activity.

Conclusions

In this study, *A. pasteurianus* JST-S was screened, identified by 16S rDNA gene sequencing, and used for the production of purple sweet potato vinegar. Orthogonal experiments showed that fermentation time, ethanol concentration, and AAB inoculum significantly ($P < 0.05$) influenced AAC. AAC reached 4.26% (v/v) under optimum conditions. Anthocyanin concentration and antioxidant activity of vinegar was evaluated; the increase in the antioxidant activity was associated with the presence of acetic acid and phenolics released upon fermentation. Future studies should evaluate the mechanism underlying the increase in the antioxidant activity caused by acetic acid and other non-phenolic antioxidants.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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