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Enhancement of Bioactive Compounds of Roselle Vinegar by Co-culture Fermentation

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Abstract

Introduction: Functional therapeutic properties of vinegar described include antibacterial activity, blood pressure reduction, antioxidant activity, reduction in the effects of diabetes, prevention of cardiovascular disease, and increased vigor after exercise. The objectives of present study were to enhance bioactive compounds and the acetic acid production of Roselle Vinegar with mixed culture fermentation. **Methods:** Vinegar fermentationis a two-step process: First, the anaerobic conversion of sugars to ethanol by *Saccharomyces cerevisiae* TISTR5048 and then the aerobic oxidation of ethanol to acetic acid by mixed culture of *Acetobacter aceti* TISTR102 and *Acetobacter cervisiae* TN4497. Total phenolic, flavonoid, anthocyanin contents and antioxidant properties of Roselle vinegar were also determined. **Results:** The Roselle wine was produced in a batch reactor. The kinetic parameters obtained for wine fermentation by *S. cerevisiae* TISTR5048 were profiled. Using mixed culture of *A. aceti* TISTR102 and *A. cervisiae* TN4497, the wine vinegar process optimization ranges found for initial concentrations of ethanol and acetic acid as independent variables were 63.47 g/L and 69.21 g/L, respectively. Bioactive compounds including total phenolics, total anthocyanins, and their antiradical activities (EC_{50}) were determined. Acetification increased total anthocyanin content, total polyphenols and antioxidant activities. The abilities of wine from Roselle juice to antiradical activity were high with EC_{50} of 14.95 mg/L. The antioxidant activity of vinegar product was high with EC_{50} of 0.72 mg/L. **Conclusion:** Acetification significantly increased bioactive compounds contents and antioxidant activities. The results indicated that fermentation is a better method for obtaining higher antioxidant activity of Roselle products.

Keywords: Roselle, vinegar, bioactive compound

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1. Introduction

Reactive oxygen species (ROS) or free radicals are generated as byproducts or interm ediates of aerobic metabolism and through reactions with drugs and environmental toxins. Although almost all organisms possess antiox idant defense and repair systems, which quench or minimize the production of oxygen-derived species, thus protecting them against oxidative damage, these protective systems are insufficient to entirely prevent the damage (Simic, 1988) caused by endogenous or exogenous oxidants (Sun, 1990). In view of this, much attention has been focused on the protective biochemical function of naturally occurring antioxidants in biological systems, and

on the mechanisms of their actions. The phenolic compounds, which are widely distributed in plants, were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems (Hertog and Feskens, 1993). The study of numerous compounds that could be useful antioxidants has generated increasing interest in the field of food or medicine. The dried flowers of *Hibiscus sabdariffa* L. (Malvaceae) commonly called “Roselle” have gained importance as local soft drink and medical herb in local regions. Studies revealed that the dried flowers of *Hibiscus sabdariffa* L., a Chinese herbal medicine, have been used effectively in folk medicine against hypertension, pyrexia, and liver disorders (Tseng *et al*., 1997). In addition in foods for centuries, white vinegar, a natural source of distilled acetic acid, provides significant and substantial health benefits when consumed in moderation. Anecdotal evidence suggests possible further astonishing benefits, including improving calcium absorption, and moderating type 2 diabetes and even some cancers. The acetic acid process was divided into two fermentation processes according to the characters of growth and metabolization of *S. cerevisiae* TISTR5048 and acetic acid bacteria: the primary fermentation for growth of *S. cerevisiae* TISTR5048 and the production of ethanol, the secondary fermentation for the production of acetic acid by acetic acid bacteria. *S. cerevisiae* TISTR5048 is facultative anaerobes, while the acetic acid bacteria is aerobe. Mixed culture systems exhibit great advantages for many processes involving more than one reaction step, such as high productivity (Mendoza *et al*., 2011).

Mixed culture fermentation may become an attractive addition to traditional pure-cultur e-based biotechnology for the traditional fermented food (Temudo *et al.,* 2008). Although the advantages of mixed culture over pure culture have been pointed out by many researchers (Harrison, 1978), little is known about the applic ation of mixed culture technology in acetic acid production (Tetsuya and Masao, 1996). In this study, mixed culture of *Acetobacter aceti* TISTR102 and *Acetobacter cervisiae* TN4497 was optimized in batch culture to enhance the yield of acetic acid production and enhance of antioxidant capacity of Roselle vinegar.

2. Materials and methods

2.1 Microorganisms

Acetobacter aceti TISTR102 and *Saccharomyces cerevisiae* TISTR5048 were from *Thailand Institute of Scientific and Technological Research* (TISTR). *Acetobacter cerevisiae* TN4497 was from Division of Biochemistry, School of Bioresources and Techonology, King Mongkut's University of Technology Thonburi, *Thailand*.

2.2 Chemicals

Gallic acid, Folin-Ciocalteu's reagent, methanol, linoleic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl, butylated hydroxytoluene, sodium carbonate, sodium hydroxide, aluminium chloride hydrated, sodium nitrite, dibasic sodium phosphate and monobasic sodium phosphate were purchased from Fluka Chemie AG.

2.3 Preparation of the Roselle mush

Dried powder (200 g) of Roselle was added with 1 L of distillated water. The physi ochemical properties of the extract were measured and recorded in Table 1. The filtrate was ameli orated using glucose to raise the sugar level to 23.7ºBrix and ammonium sulphate (111 ppm) added to stabilize the mush and to provide a nitrogen source for yeast. The pH of the mush was adjusted to pH5. The treated mush was pasteurized at 63ºC for 30 minutes and then allowed to cool to room temperature of 25ºC. The supernatant was supplemented with additional nutrients to give a base medium composition of: 1 g/L yeast extract; 2 g/L $(NH4)_2SO_4$; 1 g/L $MgSO_4 \cdot 7H_2O$.

2.4 Batch fermentation

Alcoholic fermentation

This section describes the different steps and culture media to carry out the fermen tation. In first step pre-culture, the composition of the medium was: yeast extract 10 g/L, glucose 20 g/L and peptone 20 g/L. One hundred milliliter of this YPD medium were inoculated and incu bated at 30°C under agitation (50 rpm) during 24 h. The culture medium was composed of: KH_2PO_4 5 g/L, $(NH4)_2SO_4$ 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.4 g/L yeast extract 1 g/L. The fermentation medium is a Roselle mush. It was added up to a sugar concentration of 282.36 g/L, KH_2PO_4 5 g/L, $(NH4)_2SO_4$ 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.4 g/L and yeast extract 1 g/L.

Approximately 1.0 L of Roselle mush was poured into a 2 L fermentor and inoculated with 10 ml of the *S. cerevisiae* TISTR5048 starter culture. The inoculated slurry of Roselle extract was subjected to primary fermentation at 30°C for 3 days to produce Roselle wine, which was then filtered after complete primary fermentation. Batch fermentation was conducted in a 2 L fermentor with a working volume of 1 L. The fermentation medium was inoculated with 10% v/v inoculum (20 hours culture of *S. cereviciae*, 1 x 10^7 cells/mL). The fermentation temperature was kept constant at 30 ± 0.2°C. The broth was kept under agitation at 50 rpm. Samples were taken at regular time intervals during fermentations to determine the concentrations of cell mass, etha nol, acetic acid and residual glucose in the broth.

Acetic acid fermentation

This section describes the different steps and culture media to carry out the ferme ntation. In first step pre-culture, the composition of the medium was: glucose 10 g/L, yeast extract 10 g/L, KH_2PO_4 0.5 g/L, MgSO4·7H_2O 0.5 g/L and ethanol 60 g/L. One hundred milliliter of this medium were inoculated and incubated at 30 °C under agitation (150 rpm) during 24 h. The fermentation broth was composed of: yeast extract 10 g/L, KH2PO4 0.5 g/L, MgSO4 0.5 g/L, Roselle wine (ethanol from fermentation broth 63.47 g/L). Batch fermentation was conducted in a 2 L fermentor with a working volume of 1 L. The fermentation medium was inoculated with 10% v/v inoculum (20 hours culture of *A. cerevisiae* TN4497 or *A. aceti* TISTR102, 1 x 10^7 cells/mL for monoculture fermentation; and 20 hours mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102 in ratio of 1:1, 1 x 10^7 cells/mL For coculture fermentation). The fermentation temperature was kept constant at 30 ± 0.2°C. The broth was kept under agitation at 50 rpm. Samples were taken at regular time intervals during fermentations to determine the concentrations of cell mass, ethanol, acetic acid and residual glucose in the broth. The flow chart of alcoholic fermentation acetic acid fermentation was shown in Figure 1.

Biomass estimation

Culture dry weight was measured by centrifugation and drying at 105 °C, until no weight change between consecutive measu rements was observed.

Sugar estimation

Glucose was determined by glucose (go) assay kit (Sigma Product No. G 3660)

Acetic acid and ethanol assay

Acetic acid and ethanol was deter mined by gas chromatography. An AT-Wax capillary column were used in GC system. The parameters were set as follows: carrier gas nitrogen, flow rate of carrier gas 10 mL/min, split ratio 10:1, temperature of injection port 220ºC, temperature of detector 260ºC. The column temperature program, firstly kept at 50ºC for 3 min, secondly heated up to 80ºC by the rate of 10ºC/min, then immediately heated up to 200°C by the rate of 30ºC/min and then kept at 200ºC for 1 min. Sample pretreatment was carried out as follows: 5 mL of samples were centrifuged at 15,000 g for 3–5 min. The supernatant was diluted to suitable concentration by decuple dilution and filtrated through 0.22 μm microporous membrane. Then 1.0 mL of each sample was mixed with 10 μL of isobutanol as internal stan dard. Standard sample was the mixture of 0.02% ethanol and 0.02% acetic acid. When GC worked stably, samples were injected with the volume of 1 μL.

Total phenolic content (TPC) of bioactive compound determination

The TPC was determined using a modified Folin–Ciocalteu method (Singleton and Rossi, 1965). Each test sample (250 μL) was added to a test tube that contained 6.0 ml of distilled water. After vortexing the tubes, 500 μl

of Folin–Ciocalteu's phenol reagent was added to each tube. The tubes were vortexed and 2 min later, 2.0 ml of 15% Na2CO3 was added to each tube. 1.25 mL of distilled water was added to each tube. The tubes were vortexed again and then allowed to stand for 2 h at room temperature. Thereafter, the absorbance of each sample was measured against a blank at 750 nm. A calibration curve was constructed using 50, 100, 150, 200 and 250 mg/L gallic acid as a standard. The TPC is expressed as milligrams of gallic acid per gram. Total flavonoid content of bioactive compound determination

The total flavonoid content was determined using a modified version of the method described by Zhishen et al. (1999). Each test sample (250 µl) and 1.25 ml of distilled water were added then 75 µl of 5% NaNO2 and 150 µl of 10% AlCl3 was added. After 6 min 0.5 ml of 1 M NaOH was added. The absorbance of the solution was measured against a blank at 510 nm using a spectrophotometer. A calibration curve was constructed using 0.125, 0.25, 0.5 and 1.0 g/L quercetin as a standard. The total flavonoid content is expressed as milligrams of quercetin per gram of dry extract.

Free-radical-scavenging activity of bioactive compound determination

Antioxidant activity was measured based on the scavenging of the stable free radical, 1,1-diphenyl-2-picrylhydrzyl (DPPH). A sample of each fraction (2.0 mL), in methanol, was added to 2.0 of a solution that contained DPPH. After 30 min, the absorbance was measured at a wave length of 517 nm using UV-visible spectrophotom eter.

DPPH assay

The free radical scavenging activity of different fractions was measured by the DPPH scavenging method proposed by Shimada et al. (1992). 2.5 x 10-4 M solution of DPPH in metha nol were prepared and 2.0 ml of this solution were added to 2.0 ml of different black rice extracts obtained in different storage conditions. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. The DPPH radical-scavenging activity was calculated according to the following: % of DPPH scavenging activity = {1- (AbS/AbC)} x 100, where AbC was the absorbance of the control and AbS was the absorbance in the presence of the test compound. EC50 is the effective concentration in mg extract/ ml which inhibits the DPPH activity by 50%. BHT (butylated hydroxytoluene) was used as positive control for comparison.

Anthocyanin analysis

Anthocyanin content was determined using the pH-differential method (Giusti and Wro lstad, 2001). A pigment content was calculated as cyanidin-3-glucoside and expressed as milligrams per 100 g of dry weight, using an extinction coe fficient of 26,900 $Lcm^{-1}mol^{-1}$and molecular weight of 449.2 $gmol^{-1}$.

All experiments were carried out in duplicate.

3. Results

The compositions and physiochemical properties of the Roselle extract, mush and wine were then determined, and the results of this

primary fermentation were presented in Table 1. As shown in Figure 2, the content of ethanol in culture at 84 h was 54.95 g/L while the maximum ethanol concentration (63.47 g/L) was at 72 h. Meanwhile, pH value was about 4.90, the biomass of the culture of *S. cerevisiae* TISTR5048 was promoted from 7.36 g/L at 0 h to 92.37 g/L at 84 h. The glucose was utilized by *S. cerevisiae* TISTR5048 after inoculation, and *S. cerevisiae* TISTR5048 reached the maximum growth (97.14 g/L) at 72 h.

As shown in Figure 3(A), the maximum acetic acid concentration (45.12 g/L) was at 168 h. Meanwhile, pH value was about 3.91, the biomass of *A. cerevisiae* TN4497 was promoted from 9.26 g/L at 0 h to 64.36 g/L at 168 h. The ethanol was utilized by the mixed culture after inoculation, and the mixed culture reached the maximum growth at 168 h. As shown in Figure 3(B), the maximum acetic acid concentration (49.03 g/L) was at 168 h. Meanwhile, pH value was about 3.62, the biomass of *A. aceti* TISTR102 was promoted from 9.26 g/L at 0 h to 72.14 g/L at 168 h. The ethanol was utilized by the mixed culture after inoculation, and the mixed culture reached the maximum growth at 168 h.

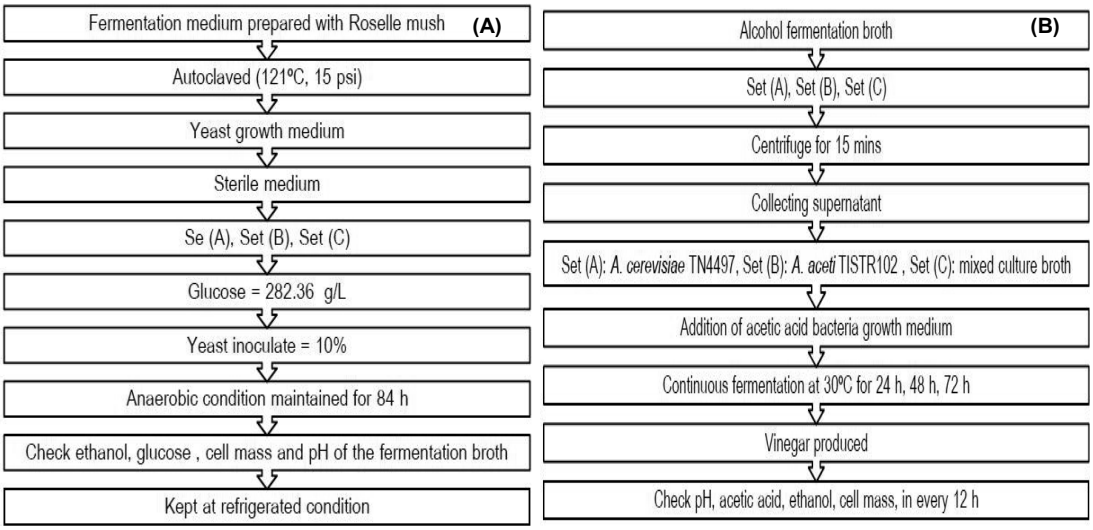

Figure 1 Flow charts of (A) alcoholic fermentation and (B) acetic acid fermentation of Roselle mush

As shown in Figure 3(C), the content of acetic acid in culture at 132 h was 59.23 g/L while the maximum acetic acid concentration (62.21 g/L) was at 168 h. Meanwhile, pH value was about 3.40, the biomass of the mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102 was promoted from 9.26 g/L at 0 h to 81.47 g/L at 168 h. The ethanol was utilized by the mixed culture after inoculation, and the mixed culture reached the maximum growth at 168 h. The maximum ethanol production (P_{MAX}), ethanol production rate (Q_E), product (ethanol) yield coefficient ($Y_{P/S}$) and

cell mass yield coefficient ($Y_{X/S}$) by *S. cerevisiae* TISTR5048 fermentation were found to be 63.47 g/L, 2.25 g/L/h 0.45 g (g-total sugar)$^{-1}$ and 1.53 g (g-cell mass)$^{-1}$, respectively. For acetic acid fermentation by the mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102, the maximum acetic acid production (P_{MAX}), acetic acid production rate (Q_E), acetic acid yield coefficient ($Y_{P/S}$) and cell mass yield coefficient ($Y_{X/S}$): were found to be 69.21 g/L, 1.15 g/L/h, 1.05 g (g-ethanol)$^{-1}$ and 1.17 g (g-cell mass)$^{-1}$, respectively. It was found that the mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102 could produce relatively high acetic acid yield (Table 2). The TPC, flavonoid and anthocyanin contents were different significantly between Roselle extract, wine and vinegar (Table 3). In the present study, Roselle vinegar, with an average TPC of 3.42 ± 0.05 mg/L, was found to possess the highest TPC among Roselle extract and wine, which were 10.22 and 2.22 times higher than that of Roselle extract (3.42 ± 0.05 mg/L) and wine (15.74 ± 0.06 mg/L), respectively (Table 3).

Table 1 The compositions and physiochemical properties of the Roselle extract, mush and wine of *Saccharomyces cerevisiae* TISTR5048

Parameter	Extract	Mush	Roselle wine
Total soluble solids (Brix)	8.2±-0.09a	23.7±0.04b	0.78±0.04c
Acetic acid (g/L)	3.27±0.06a	2.01±0.07b	6.49±0.01c
pH	4.52±0.02a	5.5±0.06b	5.2±0.02c
Glucose (g/L)	3.02±0.05a	282.36 ±0.09b	0.37±0.05c
Ethanol (g/L)	0.00a	0.00b	63.47 ±0.20c

Each value is expressed as mean ± SE (n = 3). Means with different small letters within a row are significantly different ($p < 0.05$)

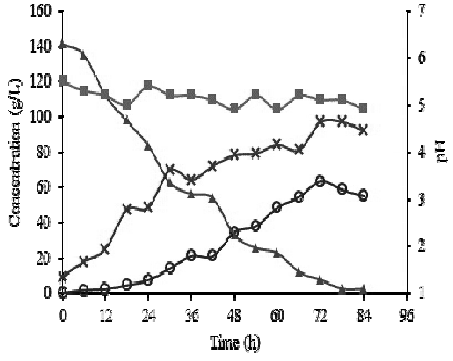

Figure 2 The time course about culture of *S. cerevisiae* TISTR5048 with batch fermentation of ethanol production (○), glucose content (▲), the cell growth (´) and pH (■)

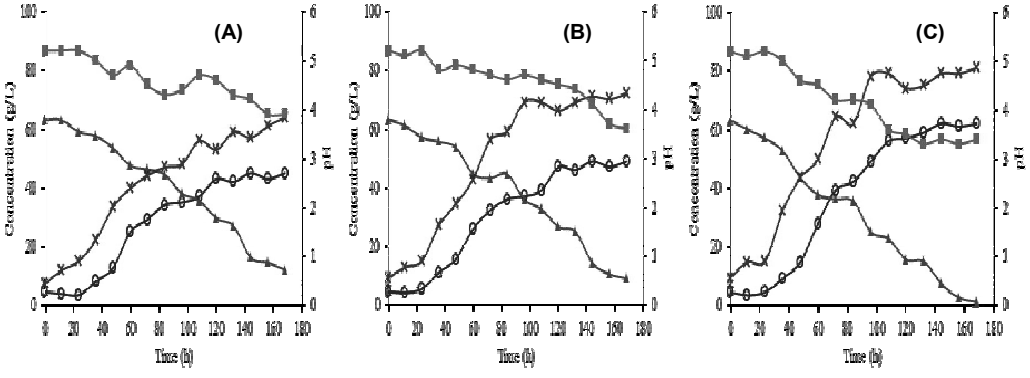

Figure 3 The time course about (A): *A. cerevisiae* TN4497, (B): *A. aceti* TISTR102 and (C): mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102 with batch fermentation of acetic acid production (○), ethanol content (▲), the cell growth (´) and pH (■)

Table 2 Ethanol production with *S. cerevisiae* TISTR5048 and acetic acid production with monoculture and mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102

Strain	P_{MAX}	Q_E	$Y_{P/S}$	$Y_{X/S}$
***S. cerevisiae* TISTR5048**	63.47	2.25	0.45	1.53
***A. cerevisiae* TN4497**	45.12	0.07	0.71	1.02
***A. aceti* TISTR102**	49.03	0.08	0.78	1.14
Mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102	62.21	0.57	0.98	1.28

Q_E : Production rate (g/L/h)

P_{MAX} : Maximum production (g/L)

$Y_{P/S}$: Product yield coefficient (g (g-total substrate)$^{-1}$)

$Y_{X/S}$: Cell mass yield coefficient (g (g-total substrate)$^{-1}$)

Table 3 Variations of bioactive compounds including TPC, flavonoid, anthocyanin contents and antioxidant their capacities of Roselle extract, wine and vinegar of mixed culture of *A. cerevisiae* TN4497 and *A. aceti* TISTR102

Roselle	TPC[a] (mg/L)	Flavonoid[a] (mg/L)	Anthocyanin[a](mg/L)	Antioxidant capacity[a,b](mg/L)
Extract	3.42±0.05a	0.54±0.02a	47.22±0.16a	43.62±0.06a
Wine	15.74±0.06b	0.92±0.01b	82.63±0.24b	32.28±0.04b
Vinegar	34.19±0.14c	3.46±0.04c	94.75±0.32c	21.79±0.07c

[a]Each value is expressed as mean ± SE (n = 3). Means with different small letters within a column at a specific anti-oxidant attribute were significantly different ($p < 0.05$)

[b]EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was scavenged by 50%. EC_{50} value was obtained by interpolation from linear regression analysis

Roselle vinegar, with an average flavonoid content of 3.46 ± 0.04 mg/L, was found to possess the highest flavonoid content among Roselle extract and wine, which were 6.40 and 3.74 times higher than that of Roselle extract (0.54 ± 0.02 mg/L) and wine (0.924 ± 0.01mg/L), respectively (Table 3). Roselle vinegar, with an average anthocyanin of 94.75 ± 0.32 mg/L, was found to possess the highest anthocyanin content among Roselle extract and wine, which are 2.00 and 1.14 times higher than that of Roselle extract (47.22 ± 0.16 mg/L) and wine (82.63 ± 0.24 mg/L), respectively (Table 3).

The functional properties of fruit juice are attributed to diverse phenolic bioactive com pounds available in the juice. These functional compounds act as antioxidant, anti-radical, anti-prolifative and lipid peroxidation inhibitor. Some researchers attributed their structural criteria to the potent biological activity of these components to free hydroxyl groups. Phenolic bioactive compounds found in fresh pomegranate juice are generally glycosylated with sugars while ferme ntation of the juice and sugar consumption by micro-organisms results in deglycosylation and release of free hydroxyl groups and relevant aglycones which can contribute to improve fun ctional properties of pomegranate (Kawaii and Lansky, 2004). In recent years, fermentation of different fruit was studied by several authors (Mousavi *et al.,* 2011; Yoon *et al*., 2006). Results revealed that since fruit juices may be served as a suitable medium to cultivate bacteria to enhance the health benefits of the food product. The free radical scavenging effect of the juice was increased through fermentation; however, the enhanced effect of fermentation varied with the employed bacteria since acetic acid bacteria exhibited higher improvement in the antioxidant activity of the juice. Improvements in the radical scavenging effect can be related to the increase in the free form of phenolic compounds and the production of other by-products through fermen tation. These findings highlight the beneficial effect of juice fermentation by the bacteria able to produce organic acids and improve the antioxidant

activity of the fruit juice. Total antioxidant activity of bioactive compounds in the Roselle vinegar was higher than that of Roselle extract and wine and a significant difference was determined (Table 3). However, the total antioxidant activities of all the Roselle vinegar were higher than that of the positive control BHT (EC_{50} of 27.30 mg/L).

The DPPH radical is a stable organic free radical with an adsorption band at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. In this study, the high DPPH radical scavenging activity of BHT and the Roselle extract, wine and vinegar were observed in a concentration dependent manner (Table 3). The Roselle extract, wine and vinegar (EC_{50}, 43.62, 32.28 and 21.79 mg/L, respectively) was more active than the positive control BHT (EC_{50}, 27.30 mg/L) Which was similar to the EC50 values for DPPH of BHT studied by Hassan et al. (2011), and Li and Chen (2012), 25.00 mg/L and 25.92 mg/L, respectively. The high DPPH radical sca venging activity of the Roselle extract, wine and vinegar were attributed to the presence of anthocyanin and acetic acid fermentation.

4. Discussion

The decrease in alcohol concentration was corresponding to the gradual rise in acetic acid concentration which accumulated from 4.63 g/L to 65.26 g/L over a fermentation progress period of 168 h by the mixed culture. Alcohol induces stress in yeast cells causing their death and flocculation, but the stress of yeast is more related to acetaldehyde which is the first interme diate product of ethanol which is oxidise to acetic acid by acetic acid producing bacteria. This acetaldehyde disrupts the enzymatic activity of yeast. The beginning of acetic acid formation is related to maximum cellular growth and sufficient biomass density to start the acetification process (Seyram *et al*., 2009).

The pH of the vinegar during the secon dary fermentation by the mixed culture was observed to decrease from pH 5.2 to pH 3.43. This initial decrease in pH provided optimal growth conditions to acetification. This fall in pH and it can be attributed to accumulation of acetic acid and other volatile short chain organic acids such as propionic, tartaric and butyric acids, which are important in development of the flavor and aroma of vinegar (Seyram *et al*., 2009).

The ethanol content continued to decrease with time from 63.47 g/L to about 0.26 g/L by 168 h. This interpretsed that the ethanol conversion to acetic acid reached zero when acetic acid reached to the maximum in the medium. The vinegar produced by the mixed cultures using the Roselle extract contained 62.21 g/L acetic acid or about 6.92 % (v/v) acetic acid and was comparable with 6.33% (v/v) and 6.11% (v/v) vinegar obtained by Vegas *et al*. (2010) in their study of two vinegar plants; Laguinelle (B, Banyuls, France) and Viticultors Masd'en gil (P, bellmunt del priorat, Tarragona, Spain). The composition and functional properties of the Roselle vinegar showed potential as a food-grade analog. Therefore, it was of interest to determine the anthocyanin contents of the Roselle, since

this was the major antioxidants in vinegar. From the results, total antioxidant activity of the Roselle vinegar was higher than that of Roselle extract and wine due to higher TPC, flavonoid and / anthocyanin contents.

Processing methods are known to have variable effects on TPC bioactive compounds and antioxidant activity of plant samples. Effects include little or no change, significant decreases or enhancement in antioxidant activity (Nicoli, 1999). Food processing can improve the proper ties of naturally occurring antioxidants or induce the formation of compounds with antioxidant activ ity, so that the overall antioxidant activity increases or remains unchanged (Tomaino *et al*., 2005). Increase in antioxidant activity following thermal treatment has been reported in tomato (Dewanto *et al*., 2002a), sweet corn (Dewanto *et al*., 2002b) and ginseng (Kang *et al*., 2006). Increase in antioxidant activity following thermal treatment has been attributed to the release of bound phenolic bioactive compounds brought about by the breakdown of cellular constituents and the formation of compounds with enhanced antioxidant activity.

Fruits and vegetables contain many antioxidant compounds, including phenolic compounds, flvonoids, anthocyanins and tocophe rols (Naczk and Shahidi, 2006). Most antioxidants are polyphenolic compounds, which act as reduc ing agents (free radical terminators), metal chela tors, singlet oxygen quenchers (Mathew and Abraham, 2006) and hydrogen donors (Miller and Rice-Evans, 1997). Fermentation is a technology used to enhance the shelf-life and nutritional and organoleptic qualities of food (Frias *et al.,* 2005). Many biochemical changes occur during fermen tation, leading to an altered ratio of nutritive and anti-nutritive components and, consequently, affect the products properties, such as bioactivity and digestibility (Zhang *et al*., 2012). Recently, this bioprocess has been applied to the production and extraction of bioactive compounds in the food, chemical and pharmaceutical industries (Martins *et al*., 2011; Torino *et al*., 2013). For example, fermentation has been applied to increase the content of phenolic bioactive compounds in legumes, thus enhancing their antioxidant activity (Lee *et al*., 2008). Torino *et al*. (2013) reported that the bioconversion of the conjugated forms of phenolic compounds into their free forms during fermentation improves their health-linked function ality. Increasing the antioxidative activity of plan t-based foods by fermentation may be influenced by various factors, including the microorganism species, pH, temperature, solvent, water content, fermentation time, kind of food and aerobic condi tions. This study provides a study of the fermenta tion that influences antioxidative activity that augment antioxidative activities in Roselle vinegar.

The most important properties of a production strain in vinegar industry are tolerance to high concentrations of acetic acid and total concentration, low nutrient requirements, inability to over oxidize the formed acetic acid, high production rate. Although a variety of bacteria can produce acetic acid, mostly members of *Acetobacter* are used commercially. Pure cultures are not widely used in the acetic acid fermentation industry (O'Toole and Lee, 2003).

5. Conclusions

In conclusion, fermentation condition influenced the level of bioactive compounds including TPC, flavoid, anthocyanin and antioxi dants in Roselle vinegar. After fermentation, the bioactive compounds and antioxidant capacity of Roselle vinegar was increased. Roselle vinegar increased in TPC, flavonoid, anthocyanin and total antioxidant activity higher than that of Roselle extract and wine. This finding is important as anthocyanin, TPC and flavonoids are potent and high value antioxidant compound and Roselle vinegar product could be the source of these compounds. From this study, the results showed that the mixed culture could shorten the fermenta tion time, reduce fermentation losses and increase the yield of acetic acid.

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