A statistical study of factors affecting natural biovinegar fermentation from pineapple peel waste

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Abstract. The objective of this work was to screen and evaluate the significant parameter which affected the natural fermentation of pineapple biovinegar. In this work, peel waste from local breed pineapple (Royal Pepina) was fermented naturally to produce an aromatic biovinegar. Full factorial design of Design Expert® was used to develop a random experimental run in which six parameters were screened off. They were the fermentation temperature (27 – 50 °C), fermentation time (5 – 28 days), fermentation condition (anaerobic and aerobic), the addition of glucose (0 – 7 %), the addition of yeast (0 – 0.3 %), and peel waste condition (slurry & juice). Three parameters were identified as significant factors, which were the condition of fermentation, fermentation temperature, and addition of glucose. The ANOVA of the model was statistically significant with R^2 of 0.9948. The pineapple biovinegar produced in this work contained 3.18 % reducing sugar, 1.03 % ethanol, 3.03 % acid, 0.61 % acetic acid, 1.43 mg equi. AA/100mL ascorbic acid, pH of 3.16, 4.0 % sucrose, 8.0 °Brix total soluble solid, and 82.06 % DPPH free-radical scavenging activity of antioxidant in biovinegar. This result stood second highest after apple cider biovinegar when compared to commercially available biovinegar.

1. Introduction

Vinegar is a sour and sharp liquid used as a condiment and food preservative. It can be made from a suitable raw material of agricultural origin, containing starch, sugars, or both, by the process of double fermentation, first alcoholic, and then acetous [1]. Vinegar may contain up to 9 % acetic acid in water and other varying types of fixed fruit acids such as citric acid, coloring matter, salts, and a few other fermentations by-products which impart a characteristic flavor and aroma of each one [1]. There are two types of vinegar in the current market which are biovinegar and synthetic vinegar. Usually, the raw material used for making biovinegar are fruits, vegetables, and cereals [1]. The synthetic vinegar is made from the chemical process derived from malt or alcohol and/or vinegar adulteration with diluted synthetic acid.

The world's demand for pineapple is increasing at approximately 5 % annually. In Malaysia, around 50 % of the pineapple produced is consumed as fresh fruits, 30 % as canned fruit, and 20 % as fruit juice [2]. During pineapple processing, the stem, crown, core, and peel are removed and discarded as waste. As much as 30 – 50 % of the total fruit weight was discarded as waste during caning [2]. So, it can be estimated more than 150, 000 kg of pineapple waste was produced each year

in Malaysia. This waste disposal can be problematic because it is high in moisture and sugar content and prone to microbial spoilage emitting foul gaseous such as H_2, CO_2, and CH_4 [3].

Biovinegar natural fermentation means utilizing the natural existence of mixed microorganism strains on the raw materials itself such as yeast and bacteria to conduct the fermentation. On the other hand, biovinegar fermentation also utilizing a specific single microorganism strain for the fermentation to occur. As known, the aroma of single strain fermentation is weaker than that of the mixed strains. The latter can increase the complexity of the aroma, variety, and concentration of volatile components. A study by Liu et al. [4] proved that apple vinegar produced using a single strain was lacking in flavour and aroma than that mixed strains. The mixed strains in fermentation produce an exquisite taste of vinegar due to the different characteristic of various microorganism. Fermentation is an effective way of harnessing the medicinal richness of fruit. The fermentation of mixed strains has been shown to produce vinegar with more health benefits than the single one. Chen et al. [5] reported higher antioxidant activity in mixed strains of citrus vinegar than its single strain by 38.3 %.

Biovinegar poses a variety of physiological functions such as antioxidant, blood glucose controller, lipid metabolism regulator, weight loss agent, and anticancer [6]. The polyphenols and melanoidins also provide the antioxidant abilities of vinegar, which resulted from the fermentation of the variety of fruits [6]. The antioxidant activities of biovinegar are derived mainly from its bioactive compounds including carotenoids and phytosterols as well as phenolic compounds, vitamins C and E [7]. A review made by Mohamad et al. [8] shows that pineapple juice vinegar, which had high total phenolic acid content, exhibited the greatest in vitro antioxidant capacity than coconut juice and Nipah juice vinegar. Compared with orange and banana, consuming pineapple can be a good sleeping inducer (melatonin) and sugar blood elevations [9].

A previous study on pineapple peel biovinegar focused on the production factors effect using one factor at a time in which much time is needed to complete all factors investigation [10]. The analysis is also limited to only one-factor contribution instead of factors interaction. A limited study on multiple factors using a statistical tool such as the full factorial design on the biovinegar production has been reported such as in the study of Selvanathan et al. [11] have investigated pineapple peel biovinegar fermentation using factorial design producing acidity around 1.12 % which was lower by 1.91 % compared to the current study. Using a full factorial design, more factors can be considered with a minimum time to finish the investigation. Analysis can include the contribution of a single factor and factors interaction. Design Expert software® can be used to achieve this purpose. Currently, very little research studied the application of full factorial design (FFD) on the production of pineapple peel biovinegar using mixed strains fermentation. In this study, FFD was applied to determine the effects of six factors on the fermentation of pineapple peel by mixed strains. Characterization of the physiochemical and free-radical scavenging activity of pineapple peel biovinegar is also rare to be found in the previous study.

2. Materials and methods

2.1. Substrate

Pekan Pina Sdn Bhd. generously supplied the pineapple fruit. The fruits were cleaned and cut to separate the peel. Cleaned pineapple peels were pureed to produce its slurry and juice at 1:1 w/v (one-part peel to one part sterilized deionized water). The slurry media was prepared by liquidized the peel using an electrical blender. The juice media was prepared by extracting the puree through a coffee filter with a pore size of 20 μm.

2.2. Screening process with Full Factorial design

The screening was performed using full factorial design (FFD) of Design Expert® software (Version 8.0.6, State-Ease). Table 1 shows the coded and actual levels of the variables. A 2^6 full factorial design consisting of 64 runs were performed for all the factors. The factors have been chosen according to previous studies. The temperature had been chosen in a range of 27 – 50 °C because it was reported

that the optimum temperature for growth of common microorganism involved in biovinegar production such as *Acetobacter* sp. and yeast fermentation was between 25 – 32 °C [12]. Moreover, it was recommended to investigate a wide temperature range for the screening study because there might exist thermophilic strains on the pineapple peel which can survive in high temperature up to 50 °C as reported by Arroyo-López et al. [12]. Furthermore, an additional carbon source might be needed when producing biovinegar. The previous study had looked into the addition of glucose to increase the biovinegar yield. A study by Raji et al. [10] reported that additional glucose up to 2.5 % and yeast into the substrate to increase biovinegar yield. In addition to the preliminary study by the authors, sugar and yeast can add up to 7 % and 0.3 %, respectively to produce higher acidity to the pineapple peel waste fermentation. As for the fermentation time, a previous study recorded a time between 11 to 40 days [10] [13]. In the current study, the author decided on the average of this range to compliment both the anaerobic and aerobic condition experienced by the microorganism in the fermentation.

Table 1. Factors and their coded and actual levels used in the method of 2^6 full factorial experiments.

No.	Factors	Coded	Type of factor	Actual values of coded levels		Units
				-1	+1	
1	Condition of media	A	Categorical	Slurry	Juice	-
2	Condition of fermentation	B	Categorical	Anaerobic	Aerobic	-
3	Temperature	C	Numerical	27	50	°C
4	Addition of glucose	D	Numerical	0	7	%
5	Addition of yeast	E	Numerical	0	0.3	%
6	Fermentation time	F	Numerical	5	28	days

A validation experiment was carried out in triplicate to validate the model suggested by the software. The experimental data were analyzed by FFD to fit the following first-order polynomial equation (1):

$$Y = \beta_0 + \sum_{\mathrm{i}=1}^{n} \beta_{\mathrm{i}} X_{\mathrm{i}} \quad (1)$$

Where Y represents the value of the response, β_0 is the constant coefficient, n is the number of variables, β_{i} represents the coefficient of the linear parameters, and X_{i} represents the coefficient of the interaction parameters.

2.3. Biovinegar fermentation set-up

Fermentation of pineapple peel biovinegar involving mixed strains was performed under an aseptic technique. The fermentation was conducted in a 100 mL serum bottle with 50 mL working volume without agitation in a batch mode. The working bottles were prepared based on suggestion composition by Design Expert® software in Table 2. Once fermentation ceased, the sample was collected and centrifuged at 8000 rpm for 15 minutes. Total acidity, in the supernatant, was estimated using the titration method. The outputs of the experimental design were analyzed with Design-Expert software to evaluate the effects of each factor involved.

Table 2. The design of the 2^6 full factorial experiments.

Standard order	Coded values of factors						Total acidity (%)
	A	B	C	D	E	F	
1	+1	+1	+1	-1	-1	+1	0.4204±0.16
2	+1	+1	-1	-1	+1	+1	0.0601±0.00
3	-1	-1	+1	+1	-1	-1	0.1802±0.00
4	-1	-1	+1	+1	-1	+1	0.2222±0.03
5	+1	+1	+1	-1	+1	-1	0.9209±0.15
6	-1	+1	+1	-1	+1	-1	0.2202±0.07
7	+1	+1	+1	+1	+1	-1	0.3803±0.03
8	-1	+1	-1	+1	-1	-1	2.0418±0.76
9	+1	+1	+1	+1	+1	+1	0.5405±0.06
10	-1	-1	-1	-1	-1	-1	0.1802±0.00
11	-1	-1	-1	+1	-1	-1	0.1501±0.03
12	-1	-1	-1	-1	+1	+1	0.2822±0.03
13	-1	+1	+1	-1	+1	+1	0.4804±0.00
14	+1	-1	-1	+1	-1	-1	0.2402±0.06
15	-1	-1	+1	-1	-1	-1	0.2102±0.03
16	+1	-1	+1	+1	+1	-1	0.3003±0.00
17	-1	-1	+1	-1	+1	+1	0.2582±0.07
18	+1	+1	-1	-1	+1	+1	0.0601±0.00
19	-1	+1	+1	+1	+1	-1	0.3803±0.03
20	-1	-1	-1	-1	-1	+1	0.2822±0.03
21	+1	+1	-1	+1	-1	+1	0.5205±0.02
22	-1	+1	-1	-1	+1	-1	0.4204±0.06
23	-1	-1	-1	+1	+1	+1	0.3903±0.04
24	+1	+1	+1	+1	-1	-1	0.5004±0.49
25	+1	-1	+1	-1	+1	+1	0.2822±0.03
26	+1	+1	-1	+1	-1	-1	1.2811±0.15
27	+1	+1	+1	-1	-1	+1	0.4384±0.09
28	-1	+1	+1	+1	-1	+1	0.3423±0.07
29	+1	+1	-1	-1	-1	+1	0.0500±0.02
30	+1	-1	-1	-1	+1	-1	0.2222±0.03

Standard order	Coded value of factors						Total acidity (%)
	A	B	C	D	E	F	
31	+1	+1	-1	+1	+1	+1	0.1802±0.06
32	+1	-1	-1	-1	-1	-1	0.2402±0.06
33	+1	-1	-1	+1	+1	-1	0.3003±0.00
34	-1	-1	+1	-1	+1	-1	0.1621±0.07
35	-1	-1	+1	-1	-1	+1	0.1982±0.03
36	+1	+1	-1	+1	+1	-1	1.9617±0.18
37	+1	-1	-1	-1	+1	+1	0.3603±0.00
38	-1	+1	+1	-1	-1	-1	0.0801±0.03
39	+1	-1	-1	-1	-1	+1	0.3003±0.06
40	+1	+1	+1	+1	-1	+1	0.3423±0.03
41	+1	+1	-1	-1	-1	-1	0.7206±0.06
42	-1	-1	+1	+1	+1	+1	0.2402±0.00
43	-1	-1	-1	-1	+1	-1	0.1982±0.03
44	+1	-1	+1	-1	-1	-1	0.3003±0.00
45	-1	+1	-1	+1	+1	-1	1.5013±0.24
46	-1	-1	-1	+1	+1	-1	0.2702±0.04
47	+1	+1	+1	-1	+1	+1	0.5225±0.03
48	+1	-1	-1	+1	+1	+1	0.3903±0.04
49	+1	-1	+1	-1	+1	-1	0.1802±0.00
50	-1	+1	+1	+1	+1	+1	0.4384±0.03
51	+1	-1	+1	+1	-1	-1	0.2402±0.00
52	-1	-1	+1	+1	+1	-1	0.2582±0.03
53	-1	+1	+1	+1	-1	-1	0.2602±0.03
54	-1	-1	+1	+1	-1	+1	0.1802±0.06
55	+1	-1	+1	-1	-1	+1	0.2402±0.00
56	+1	+1	+1	-1	-1	-1	0.2202±0.03
57	-1	+1	-1	+1	-1	+1	1.0109±0.18
58	+1	-1	-1	+1	-1	+1	0.3003±0.00
59	-1	+1	-1	-1	-1	-1	1.3211±0.21
60	-1	+1	-1	-1	-1	+1	0.0400±0.02
61	-1	+1	-1	+1	+1	+1	2.7804±0.25
62	-1	-1	-1	+1	-1	+1	0.3003±0.06
63	+1	+1	-1	-1	+1	-1	0.6205±0.07
64	+1	-1	+1	+1	+1	+1	0.3003±0.00

2.4. Analytical methods

2.4.1. Total acidity, pH, the content of sugar, total soluble solids, and ethanol analysis. The total acidity was estimated using the titration method were 1.0 mL biovinegar, phenolphthalein, and a neutralizing agent of 0.1 M NaOH were used and yielded total acid content (%). The titration analysis was performed in triplicate on each sample. This method was adapted from Raji et al. [10]. Total acidity (%) was estimated using equation (2).

$$\text{Total acidity} = \frac{\text{Number of moles NaOH}}{\text{Volume of diluted sample}} \times \text{Dilution factor} \quad (2)$$

A pH meter (Mettler Toledo) was used for all pH value measurements. The sucrose, alcohol and total soluble solids (TSS) content were estimated using a refractometer (MASTER-KMW 2593, Atago, Japan).

2.4.2. Acetic acid quantification. The acetic acid was quantified using HPLC (Agilent Technologies, Palo Alto, CA, USA) adopted a method by Zhang et al. [14] with modification. Samples were filtrated with a 0.45 µm membrane filter. For acetic acid analysis, a Synergy Hydro C18 250 organic acids column (300 × 4.6 mm, Japan) with sulphuric acid at 0.5 mL/min, measured with a UV detector at 221 nm (1260 VWD, 1200 series; Agilent Technologies).

2.4.3. Reducing sugar analysis. The reducing sugar was estimated using dinitrosalicylic acid (DNS) method of Teixeira et al. [15]. 1.5mL of biovinegar was added into 3 mL DNS reagent and the mixtures were heated at 100°C for 5 min. After cooling to room temperatures, 0.2 mL was withdrawn and diluted with 1.8 mL citrate buffer. Each sample was scanned with a UV/VIS spectrophotometer at wavelength 540 nm to obtain the OD values and compared with the glucose calibration curve.

2.4.4. DPPH free radical scavenging activity, and ascorbic acid content. The DPPH free radical scavenging activities of various samples were measured using the method of Thaipong et al. [16]. DPPH solutions (6.09×10^{-4} mol/L) were prepared in methanol. 2, 850 µL of fresh DPPH working solution was mixed with 150 µL of each of biovinegar sample. The mixtures were stored in the dark for 30 min at room temperature without agitation. After the reaction, the absorbency was measured at 515 nm using UV/Vis spectrophotometer. A known antioxidant, *i.e.* ascorbic acid, was used to develop the free radical scavenging calibration graph. The concentration of ascorbic acid was determined by a using radical scavenging calibration graph. DPPH solution and methanol were used as the control and blank, respectively. All tests were performed in triplicate. The percentage of free radical scavenging activity was calculated according to equation (3):

$$\%\text{DPPH radical scavenging activity} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100\% \quad (3)$$

3. Results and discussion

3.1. Statistical analysis for acid production from pineapple peel biovinegar

Table 2 shows the total acid concentration obtained from natural fermentation was between 0.05 to 2.78 %. The highest content of acid (2.78 %) was obtained from 28 days of fermentation at 27 °C, using pineapple peel slurry as media, 7.0 % glucose and 0.3 % yeast in aerobic condition as shown in standard order 61.

The analysis of variance (ANOVA) was done to determine the significance of the model suggested by the software. The statistical significance of regression can be determined using *F*-values, while the *p*-values were used to examine the significance of each coefficient as shown in Table 3. From the

model, *F*-value is 50.83 shows that only a 0.01 % chance that the model *F*-value of this large could occur due to noise [17]. Low *p*-value ($p < 0.0001$) showing the significance of the corresponding factor [18]. The model term effect of *A*, *B*, *C*, *D*, *E*, *F*, *AB*, *AD*, *AF*, *BC*, *BD*, *BF*, *CD*, *CF*, *DE* and, *EF* was statistically significant in affecting the acid production.

Table 3. Test of significance for regression coefficient.

Source	Sum of squares	Mean square	*F*-value	*p*-value*	
Model	15.73	0.31	50.83	< 0.0001	significant
A-Condition of media	0.054	0.054	8.72	0.0112	
B-Condition of fermentation	2.60	2.60	419.77	< 0.0001	
C-Temperature	1.19	1.19	192.65	< 0.0001	
D-Addition of glucose	1.06	1.06	171.06	< 0.0001	
E-Addition of yeast	0.098	0.098	15.87	0.0016	
F-Fermentation time	0.21	0.21	34.72	< 0.0001	
Residual	0.080	$6.19\times10^{-0.003}$			
Cor Total	15.82				

R^2 = 0.9948. **p*-values greater than 0.05 indicating the model terms are not significant.

The satisfactory R^2 value of 0.9948, indicates the best model fits the experimental values and predicted well. The final equations in term of coded factors were determined as shown in equation (3):

$$Y = 0.46 + 0.029A + 0.20B - 0.14C + 0.13D + 0.039E - 0.058F + 0.050AB - 0.080AC + 0.056AD + 0.054AF - 0.12BC + 0.12BD + 0.019BE - 0.086BF - 0.13CD + 0.078CF + 0.039DE + 0.035EF - 0.077ABC + 0.055ABD + 0.049ABF - 0.040ACD - 0.024ACF + 0.037ADE + 0.041ADF + 0.064AEF - 0.12BCD + 0.10BCF + 0.019BDE + 0.025CDE - 0.050CDE - 0.016CDF - 0.039CEF \quad (3)$$

Where *Y* is referred to as the response of acid yield, *A* is a condition of media, *B* is a condition of fermentation, *C* is temperature, *D* is the addition of glucose, *E* is the addition of yeast and *F* is fermentation time. Factors of *A*, *B*, *C*, *D*, *E*, and *F* are referred to as the main effect, while *AB*, *AC*, *AD*, *AF*, *BC*, *BD*, *BF*, *CD*, *DE*, and *EF* are the interaction effects.

3.2. Contribution of main factors and its interaction

Two-level factorial design analysis able to determine factor contribution in the biovinegar production. Table 4 shows the contribution of the main factor and its interaction with acid production. The highest contributor was factor B, condition of fermentation either aerobic or anaerobic, with a 16.43% contribution. Based on the previous studies by Raji et al. [10] and Karazhiyan et al. [19], both were carried out the two-stage fermentation, anaerobic fermentation to produce alcohol, followed by aerobic fermentation for acid conversion with acid production between 4.77 and 5.30 %. In the two-stage fermentation, it took around 11 days for the acid to produce [20]. Some also skipped the alcohol production and performed aerobic fermentation using pineapple wine from peelings as reported by Roda et al. [13] with 5 % of acid production. However, in the current study, the condition was distinctively separated to evaluate this effect on the fermentation time and acid production. Acid production recorded by the current study was 2.8 % provided it used the natural microorganism compared to the previous study which added specific microorganism to boost the acid production. It took 28 days to complete the fermentation.

Table 4. Percentage contribution of main factor and their interaction

Factor	Contribution (%)
Condition of fermentation (B)	16.43
Temperature (C)	7.54
Addition of glucose (D)	6.70
AC	2.57
AD	1.27
BC	5.45
BD	5.56
BF	2.99
CD	6.79
CF	2.48

The second highest contributing factor was the temperature, with 7.54 %. A study by Sussou et al. [21] and Roda et al. [13] reported that temperature has to be kept in the range between 25 and 30 °C in which is the optimum condition of yeast and *Acetobacter*, thus highlighted that temperature was found to affect the fermentation of alcohol and acetic acid. The optimum temperature for biovinegar production by *Saccharomyces cerevisiae* was recorded between 13.9 and 34.1°C [12].

Another factor also considered as significant was factor D (addition of glucose) with a percentage contribution of 6.70 %. Glucose is used by microorganisms for both biosynthesis and energy production. However, high sugar concentration might decrease the growth and viability of microorganisms due to osmotic stress [22], thus the optimum level of glucose concentration is important to be determined. In a study by Raji et al. [10], the optimum level of glucose was 2.5% which lower than the current study.

The interaction between the main factors shows nine significant interactions that affected acid production. Interaction between temperature and addition of glucose was the highest among them with 6.79 % contribution. Another interaction of the main factor was mainly revolved between factor A (condition of media), B (condition of fermentation), C (temperature), D (addition of glucose), E (addition of yeast), and F (fermentation time).

3.3. Validation of model

The model suggested by DOE was validated experimentally through a triplicate run. The error obtained was 13.04% with acid production at 2.34 % within the studied range. The error between the experiment and predicted data is below 20% thus it is an acceptable error for the biological experiment. Two factors (temperature and addition of glucose) were selected as the significant factors on acid production. The result was 40% higher than that produced by Roda et al. [23] using the same raw material as the latter used single strain. Besides, the use of mixed strain fermentation can increase the complexity of the aroma and increase the variety and concentration of volatile components [3].

3.4. Physicochemical parameters of pineapple peel biovinegar

The physiochemical parameters and free radical scavenging activity have been carried out on pineapple peel biovinegar alongside two commercial vinegar commonly used by Malaysian; dates and apple cider vinegar.

3.4.1. Total acidity, pH, the content of sugar, total soluble solids, and ethanol analysis. The pH values of various biovinegar were ranged between pH 2.73 and 3.16, with pineapple peel biovinegar having the highest value as shown in Table 5. Total acidity ranged between 3.03 and 5.83 % with the pineapple peel biovinegar having the lowest value. Kim et al. [24] reported similar findings; the pH of commercial vinegar in Korea was from pH 2.81 to 3.20 with acid content up to 2.41 %. Xia et al. [25] reported that the total acidity was between 3.5 and 8.0 % in Shanxi aged vinegar. In a study by Roda et al. [13] the total acids produced by the pineapple peel and core was 5.0 %. Raji et al. [10] reported

around 4.77 % of total acid content in biovinegar made by thin strips of pineapple peel. By comparing the data of total acidity in different types of biovinegar, the current study biovinegar has 20 % higher acid than that in Korea vinegar, 90% lower than the Shanxi aged vinegar. Meanwhile, the ethanol content of the pineapple peel biovinegar was 1.03 % in which was the lowest of other biovinegar. As for the non-reducing sugar; i.e. sucrose, the concentration for the current study biovinegar was 4.0 % in which the highest with total soluble solid (TSS) of 8.0 °Brix. The TSS may include soluble solids such as sugar, organic acid, amino acids, and soluble pectin.

Table 5. Total acidity, pH values, the content of sucrose, ethanol and total soluble solids

Biovinegar	Acidity (%)	pH	Sucrose (%)	Ethanol (%)	Total soluble solid (°Brix)
Pineapple peel	3.03±0.03	3.16±0.09	4.00±0.25	1.03±0.15	8.0±0.25
Apple cider	5.34±0.76	3.12±0	1.75±0	1.70±0	3.5±0
Dates	5.83±0.59	2.73±0	1.50±0	1.50±0	3.0±0

3.4.2. Acetic acid content. The acetic acid content of various biovinegar is shown in Table 6. The pineapple peel biovinegar had low acetic acid content. Commonly, acetic acid is the main acid in the biovinegar. Zhang et al. [26] reported an acetic acid present in Zhenjiang Aromatic vinegar was 6.61 %. Biovinegars also contain volatile and non-volatile acids. A study by Zhang et al. [26] around 27% of non-volatile acids presents significantly as lactic acid while the rest of organic acid most probably volatile acids which might sum up to around 73%. The possible volatile acids present are acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid and, pyroglutamic acid [26].

Table 6. Acetic acid and reducing sugar content.

Biovinegar	Acetic acid (%)	Reducing sugar (%)
Pineapple peel	0.61±0.05	3.18±0.50
Apple cider	3.91±0.05	0.31±0.07
Dates	4.91±0.01	0.25±0.03

3.4.3. Reducing sugar content. Reducing sugar present in biovinegar is shown in Table 6. The pineapple peel biovinegar contained the highest reducing sugar of 3.18 %. Fructose and glucose are both present the highest in the fruit vinegar, with content varying by types of fruits used [24]. In this case, pineapple commonly has 2.40 % glucose and 2.15 %fructose in its flesh.

3.4.4. DPPH free radical scavenging activity, and ascorbic acid content. The DPPH free radical scavenging activity of various biovinegar is shown in Table 7. The pineapple peel biovinegar was the second highest with anti-free radical activity (82.06 %) which is the efficiency to scavenge half of the radicals. Kim et al. [24] had assessed the antioxidant activities of commercial vinegar drinks using DPPH assay and found out that blackberry vinegar has 12.07 % anti-free radical activity. Sakanaka and Ishihara [27] reported that the persimmon Saijo had 84.2 % radical-scavenging activity on DPPH radicals. The result from current shows that biovinegar are also free radical-scavengers, particularly of the peroxyl radicals, which are the major propagators of the oxidation chain of fat, thereby terminating the chain reaction [27].

Table 7. Radical scavenging activity and ascorbic acid content

Biovinegar	DPPH (%)	Ascorbic acid (mg equi. AA/100mL)
Pineapple peel	82.06±0.60	1.43±0.10
Apple cider	85.55±0.06	1.49±0.01
Dates	24.95±0.68	0.44±0.12

Antioxidants may reduce the risk of many diseases, including heart disease and certain cancers. Antioxidants scavenge free radicals from the body cells and prevent the damage caused by oxidation. Antioxidant activity can be speculated from the ascorbic acid content. The ascorbic acid in the current pineapple peel biovinegar was 1.43 mg equi. AA/100mL as shown in Table 7. The pineapple peel biovinegar stands the second-highest after apple cider vinegar. Ascorbic acid is also known as vitamin C in the common term. Such results were also been reported by Kong et al. [28] with 2.32 mg/100mL ascorbic acid produced by fermented papaya beverage. As ascorbic acid is heat-sensitive; fermentation, pasteurization, and sterilization had resulted in its reduction compare with the fresh juice although fermentation led to the retention of ascorbic acid in the vinegar studied [29]. Ayub et al. [30] reported a maximum of 26 % decreased level of ascorbic acid during pasteurization of strawberry juice. Vitamin C is known for its strong antioxidant that may reduce the risk of chronic diseases. Furthermore, may help battle high blood pressure and fights heart disease risk factors. The common shelf life of ascorbic acid is three years, and to prolong the effectiveness best to avoid heat and airflow.

4. Conclusion

Factorial screening of biovinegar utilizing pineapple waste by natural fermentation was successfully conducted and produced an acidity of 3.03 %. The full factorial screening was able to determine the effect of the six factors on acid production. Factor condition of fermentation contributed the most by 16.43 % for the acid production followed by temperature and addition of glucose with the contribution of 7.54 % and 6.70 %. Based on the ANOVA, the model was statistically significant with R^2 of 0.9948. On top of that, the pineapple peel biovinegar was comparable to commercial biovinegar based on the physicochemical properties. It is also successfully notified that pineapple peel biovinegar does have high DPPH radical scavenging activity and ascorbic acid content comparing to apple cider which scores the highest only by 3.49 % and 0.06 % differences. In the future, it is best to optimize the significant parameters that have higher contribution using response surface method (RSM) to improve the acid production.

5. References

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