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Effect of acid pre-treatment and two-stage oxygen-assisted fermentation on the production of vinegar from lignocellulose biomass peel of pineapple

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The valorization of lignocellulosic waste stands as a promising avenue to bolster sustainable food production and consumption within a circular economy framework. This study centered on the production of vinegar from pineapple peels through a two-stage fermentation process aided by oxygen. The pineapple peels underwent sorting, washing, drying, and subsequent grinding into a powder. This powder was subjected to hydrolysis using dilute sulphuric acid, followed by primary alcoholic fermentation utilizing Saccharomyces cerevisiae. The resulting fermented must was then subjected to oxidation in a second stage, facilitated by Acetobacter aceti, with varying concentrations of oxygen. A central composite design involving three factors, fermentation time, bacteria inoculum, and oxygen was employed to investigate the impact of these process parameters on the physicochemical attributes (pH, specific gravity, total soluble solids, titratable acidity) and the ferric reducing antioxidant power (FRAP) of the produced vinegar. The acid hydrolysis phase led to a notable rise in total soluble sugars (6 to 11.5 oBrix) and glucose concentration (300 to 580 mg/dL). Primary fermentation resulted in significant reductions in pH (7.02 to 5.38), total soluble solids (11.5 to 6 oBrix), and glucose concentration (580 to 62 mg/dL), accompanied by marked increases in titratable acidity (g/100 ml) and alcohol content (0.6 to 7%). The volume of oxygen demonstrated significant effects on acetic acid content, pH, and specific gravity, with the highest values (4.68 g/100 ml, 4.02, and 1.004, respectively) achieved at the maximum oxygen volume of 100 ml. The FRAP values ranged from 16.7 to 24.97 mg Fe2+ / mg, with the sample lacking oxygen displaying the highest FRAP. Furthermore, fermentation time and bacteria inoculum exerted significant effects on acetic acid content, with an optimal value of 4.43 g/100 ml. Interaction between bacteria inoculum, oxygen volume, and fermentation time also had significant effects on specific gravity.

Key words: Lignocellulose waste-biomass, Pineapple peel, Acid Pre-treatment, Two-stage Fermentation, Vinegar.

INTRODUCTION

The growing concern over sustainable food waste management has gained momentum, particularly in the context of the UN Sustainable Development Goals (UN-SDG). The integration of effective waste management practices, responsible production, and consumption aligns with achieving the objectives of the UN-SDG

framework (Rodić and Wilson, 2017; Lemaire and Limbourg, 2019; Pujara et al., 2019). This convergence highlights the potential for linking waste management concepts with the promotion of good food waste practices. Recent efforts have been dedicated to converting agro-food waste into valuable products, driven by the principles of sustainability (Cheok et al., 2016; Ong et al., 2018; Sindhu et al., 2019; Rico et al., 2020). Lignocellulosic waste-biomass, such as the one discussed in this study, holds promise as an economically viable and readily accessible renewable bioresource, capable of yielding useful bio-products (Bhatia et al., 2020). Among the bio-products derived from lignocellulose waste, vinegar has been successfully produced through fermentation, utilizing various lignocellulosic sources including fruit peels (Roda et al., 2014), which are abundantly consumed worldwide. Consequently, a substantial volume of fruit waste, particularly fruit peels, accumulates (Su et al., 2016). Pineapple (*Ananas comosus*) and other fruit peels, recognized as common fruit waste in Cameroon, illustrate this pattern. Approximately 80% of pineapple components, including peels, crowns, leaves, core, and stems, are discarded during processing, transportation, and storage, ultimately becoming waste (Roda and Lambri, 2019; Zainal Alam et al., 2020). Studies reveal that pineapple peels contain sugars that, although not readily accessible (Roda et al., 2016), can be converted into fermentable forms for producing ethanol and other bio-products (Lucarini et al., 2021) with appropriate pre-treatment. This underscores the potential for transforming waste materials into valuable resources, aligning with sustainability goals and addressing the challenges of food waste.

The main compounds found in lignocellulose biomass are cellulose (35-50%), hemicelluloses (25-30%), and lignin (25-30%) (Anwar et al., 2014). The cellulose and hemicellulose are densely packed in layers of lignin, which hinders the biological digestibility of the cellulose present in lignocellulose biomass (Mosier et al., 2005). This has made the hydrolysis process rate-limiting thereby requiring prior biomass pre-treatment in order to disrupt the lignin layers, thereby exposing the cellulose and hemicellulose, and therefore increases the yield of sugars (Mosier et al., 2005; Kumar et al., 2009).

Acids have been used in biomass pre-treatment with some improvements in the digestion performance. The most common acid in this process has been sulphuric acid, which have been documented to be very potent in the total removal of the hemicellulose component of corn stalk, whole corn stalk, Sorghum stalk, grasses and other lignocelluloses after pre-treatment (Di Cai et al., 2016; Yangyang et al., 2017; Xiaoling et al., 2018). Many raw materials have been use in the production of vinegar, but pineapple peels are promising as a renewable fermentation substrate due to their high sugar contents, up to 8.92% (Ali et al., 2020). Production of vinegar is mostly carried at home scale or cottage industries using natural fermentation (Ezenekwe et al., 2021). In the modern commercial production of vinegar, the generator method and the submerged fermentation method are used. These methods are based on the goal of infusing as much oxygen as possible into the alcohol product to speed up the acetic acid fermentation process. The acetification of alcohol to vinegar from using direct oxygen may have different physicochemical properties. Yet no studies have been available for use of pure oxygen for vinegar fermentation of pineapple peels biomass.

With an increasing demand for vinegar, necessitating the development of an eco-friendly process (Gunjan and Haresh, 2020), it is necessary to optimize the technologies used (Piotrowski and Kubica, 2021). Oxygen assisted fermentation is an invention in the fermentation process wherein substantially pure oxygen is directed into the fermentation medium. Oxygen makes up about 20% of normal atmospheric air (Zheng et al., 2018). When correctly applied, oxygen interacts with both the microorganism in such a way that its health is improved and fermentations encounter less problems (Shea, 2018). The use of oxygen assisted fermentation has been shown, not only to reduce energy consumption but also reduces cycle of time used for the process and reduces contamination of product. The main objective of this study is to evaluate the use of oxygen assisted two-stage solid fermentation and effects of process parameters on the quality of vinegar produced.

MATERIALS AND METHODOLOGY

Collection of raw material

Pineapples peels were gotten from fruit vendors in the Bamenda food market. They were sorted to remove damaged peels, washed with tap water and cut into smaller pieces in order to facilitate drying. The peels were dried in an oven at 60°C for 48 h (Pereira et al., 2022). The dried peels were then ground and sieved using 250 um pore size sieve. The pineapple peels powder served as sample (Casabar et al., 2019).

Acid hydrolysis of sample

Acid penetrates lignin without any preliminary pretreatment of biomass, breaking down the cellulose and hemicellulose polymers to form simple sugar molecules (Lenihan et al., 2010; Satyanagalakshmi et al., 2011). The biomass was mixed with dilute sulphuric acid (H_2SO_4) in the ratio 1:5. The mixture was placed in an autoclave at 121^{o}C and a pressure of 1 bar for 15 min after release of first pressure. The pH of the sample was adjusted using

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a NaOH base. The total soluble solids, glucose level and specific gravity were recorded.

Fermentation

Activation of yeast (Saccharomyces cerevisiae) and inoculation for fermentation

In order to ensure the viability of the yeast and to reduce the duration of the lag phase during fermentation, 10g of the yeast was added into a litre of fresh must and allowed at 30°C. The sample was observed after every 15 min until development of bubbles signifying the start of fermentation and release of CO_2. At this state, it was then used as inoculum during fermentation for wine production. Prior to inoculation, must samples were pasteurised in order to eliminate all vegetative forms of microorganisms. The inoculum was then applied. This phase of fermentation was carried out anaerobically for 96 h at 30°C.

Preparation of bacteria culture for acetic acid fermentation

Acetic acid bacteria were isolated from palm wine. To do this, palm wine put in a sterilized container at room temperature until fruit flies were seen. Culture media was prepared which include: yeast extract, standard medium and enrichment medium, using the different culture techniques. The cultured samples were inoculated on GYC medium and incubated at 37°C.

Preparation of glucose yeast calcium carbonate agar GYC medium (standard medium)

GYC medium is made up of different quantities of reagents (5% glucose, 1% yeast extract, 1% $CaCO_3$ 1.5% agar). Using an electronic balance, 85 g of the medium was measured and dissolved in 1 L of distilled water. It was well mixed then heated until boiling for 1 min to ensure complete dissolution. The solution was cooled to 45-50 ºC and aseptically 70 ml of ethanol was added (Mizzi et al*.,* 2022).

Preparation of enrichment medium

1% mannitol, 0.5% ethanol, 0.3% acetic acid, 1.5% peptone, 0.8% yeast extract were weighed using an electronic balance and diluted in 100 ml of distilled water. The solution was transferred into a sterile bottle and autoclaved at 115°C for 15 min.

Inoculation and incubation of samples

Isolation of *Acetobacter aceti* was accomplished by inoculating the sample, using the pouring method, on standard GYC medium. In an inverted position, the petri dishes were incubated at 37°C, for 48 h. The yellow colonies that developed were sub cultured by streaking on a new media for 48 h. Each distinct bacteria colony was then picked for identification.

The colony characteristics including color, size, shape and elevation were studied after incubation. In order to further identify the bacteria, the cells were tested for their gram reaction, catalase activity, oxidase activity, cell shape and scent. Also, the optical density of the bacteria was measured in order to estimate the concentration of acetic acid bacteria in the culture medium.

Purification of culture isolates

All the colonies, which appeared after 48 h of incubation on the surface of standard medium GYC plates, were examined for morphological characteristics. The required *Acetobactor aceti* colonies were inoculated on the surface of standard GYC medium for specific culture isolates until pure growth was obtained. Then one loop of cells from the pure culture was inoculated into the enrichment medium and *Acetobactor aceti* growth medium was prepared and ready for inoculation into fermentation broth samples.

Optimization of the acetification fermentation process

Prior to acetification, the alcoholic fermented samples were heated to 70°C to kill yeast. *Acetobactor aceti* inoculum was added after cooling to 33°C, temperature at which acetic acid fermentation was carried out.

Effect of two stage oxygen assisted fermentation on the physicochemical and antioxidant properties of vinegar produced

Under conditions of pineapple peel vinegar fermentation temperature of 33°C, 15% inoculation of acetic acid bacteria, 7% alcohol and oxygen saturation of 20, 40, 60, 80 and 100% (Table 1), were investigated the effect of the amount of oxygen on the physicochemical and antioxidant properties of vinegar evaluated. This was compared with samples with no inoculum and no oxygen and that with inoculum but no oxygen.

Experimental design

The experimental design used by Debapriya et al. (2019) and Guo et al. (2018) was modified and applied. Three factors were considered for optimization (oxygen (60-120%), fermentation time (3-7 days) and acetic acid bacteria inoculum (10 -20%) using a two level Central Composite Design (CCD) and Response Surface Methodology (RSM) with coded values A, B, and C, respectively, as shown in Table 2. The acetic acid concentration in the fermentation broth (g/100ml) was analyzed as the main response. Other responses included, ferric reducing antioxidant power (FRAP), pH, Brix, specific gravity and temperature. A total of 16 sets of runs were generated using STATGRAPHICS. Analysis of variance (ANOVA) was used to test the effect of the different factors on response variables and the interaction between the responses and the interaction variables examined using model equations. Error sum of squares (SSE), regression sum of squares (SSR) and corrected sum of squares (SST) were determined using ANOVA analysis. The coefficient of determination, R^2 expressed the polynomial model's fit quality. A confidence level of 95% was presumed to be significant in this study. Based on the effect of three factors, the respective Pareto plots were obtained for both levels.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + E \quad (1)$$

Analysis of vinegar

Physicochemical analysis of samples

Specific gravity: A hydrometer (IP67) was used to measure the specific gravity by dipping directly into the sample. The meter was read and results recorded.

Measuring TSS: Three drops of each sample was placed on the prism of a brix refractometer (RETK-78), such that the sample

Table 1. Variation of oxygen volume/percentage.

Sample	Oxygen saturation (%)	Bacteria inoculum (ml)
1	20	15
2	40	15
3	60	15
4	80	15
5	100	15
6	0	15
7	0	0

covers the entire prism surface. The cover plate was closed and allowed for 30 seconds then TSS was read from the eye piece on the refractometer using the eye. TSS was expressed as brix degree indicating the mass in gram of dry matter for 100g.

Determination of acetic acid content (San, 2005): The acidity was determined by titration as described by Chalchisa and Dereje (2021) with 0.1N NaOH and phenolphthalein as indicator. 0.1ml of phenolphthalein (0.05%) was added to 5 ml sample of vinegar plus 20 ml distilled water in a 250 ml dry conical flask. The titrated volume was noted as soon as the endpoint, a steady pink coloration, was reached.

Determination of pH: The pH of each sample was measured directly using a digital pH meter calibrated with buffer solutions of pH 6.86, 4.01 and 9.18 respectively at 25°C.

Determination of alcohol content: At the end of the alcoholic fermentation, an alcometer was used to measure the percentage alcohol.

Density (**Ademiluyi and Mepha, 2013**)**:** An empty 25 ml pycnometer was weighed and recorded using an electronic balance. The sample was then poured into the pycnometer and covered with the glass cover of the pycnometer. Overflow of the liquid was cleaned and the weight of the pycnometer plus sample was weighed. The mass of the sample was gotten by subtracting the weight of the empty flask from that of pycnometer plus sample. The density (g/ml) was calculated as a ratio of the mass of sample (g) to the volume (25 ml) of the pycnometer.

Determination of antioxidant activity of vinegar: Ferric Reducing Antioxidant Power (FRAP) Assay. 1 ml of sample was placed in a sterilized glass tube and 2.5 ml buffer solution (0.2M Phosphate buffer, pH 6.6) added. 2.5 ml of 1% potassium fericyanide solution was added and vortex. It was then incubated at 50°C for 20 min after which 2.5 ml of 10% TCA acid (Trichloro acetic acid) was added. The resulting mixture was centrifuged at 3000 rpm for 10 min and 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water, then 0.5 ml of 0.1% $FeCl_3$ was added to obtain a blue colour, and the absorbance read at 593 nm against a blank. The absorbance read was compared with the standard calibration curve (Vijayalakshmi and Ruckmani, 2016; Wilawan et al., 2019).

Statistical analysis

The experiments were conducted in triplicates and the mean values calculated. The statistical analysis was performed using STATGRAPHICS centurion version XVII. Analysis of Variance (ANOVA) and probability value (p-value) ≤ 0.05 was considered as a significant difference. R^2-value >70 and/or standard error <10 was used for model validation.

RESULTS AND DISCUSSION

Physicochemical properties of pineapple peels

The pH, TSS, specific gravity and sugar concentration of pineapple peels before and after pre-treatment are presented on Table 3. The sugar concentration was higher than the meter could record after pre-treatment. It was then diluted with distilled water to 580 mg/dl. The pH of neutralization was 7.02. The alcohol content after pre-treatment was 7%.

Acetic acid bacteria culture and identification

Figure 1 shows the acetic acid bacteria growth on a GYC medium (A) and microscopic view after gram staining (B). The morphological characteristics are presented on Table 4. Acetic acid bacteria were able to grow on GYC medium, had a rod shape, flat elevation, smooth surface, gram negative, catalase positive, oxidase negative, looked whitish. The results were similar to that obtained by Mathew et al. (2019), who isolated acetic acid bacteria from previous batch of apple cider vinegar. The different characteristics of the bacteria culture demonstrated that it belonged to *Acetobacter* genus. Results obtained were also similar to that of Zahoor et al. (2006).

Bacteria viability

The optical density obtained at 600 nm was measured in order to check the viability of bacteria cells in the medium. The optical density increased steadily within 72 h, then decreased (Figure 2). Increase in optical density

Table 2. The experimental design matrix with real variables.

Sample	A	B	C
1	120	3	10
2	90	5	15
3	120	7	20
4	39.5462	5	15
5	60	7	10
6	120	3	20
7	60	3	10
8	90	8.36359	15
9	90	5	23.409
10	90	5	6.59104
11	60	7	20
12	90	1.63641	15
13	90	5	15
14	140.454	5	15
15	60	3	20
16	120	7	10

The general quadratic model with three factors and interactions is depicted in Equation 1.

signifies cell viability. The decrease after 72 h could be due to nutrient depletion in the medium.

Effect of oxygen assisted two stage fermentation on the physicochemical and anti-oxidant properties of vinegar

Effect of oxygen assisted fermentation on pH

As shown in Figure 3, there was a drop in pH as fermentation time increases, with values ranging from 5.38 on day one to 4.06 on day six, with the lowest value of 4.06 observed in sample 5. Samples 6 and 7, which developed in the absence of oxygen, showed no difference in pH throughout the experiment. Even though sample 6 was inoculated with bacteria, there was no effect on the pH as compared to the other samples. Samples 1 to 5 showed a reduction in pH, with sample 5 recording the lowest pH. This indicates that increasing the amount of oxygen leads to a decrease in pH, which could be attributed to the fact that alcohol in the samples was being converted to the weak acid, acetic acid, by the bacteria present. Decrease in pH was in line with the findings of Ezenekwe et al. (2021) on production and physicochemical evaluation of vinegar produced from pineapple and pawpaw fruits with their peels. Raji et al. (2012) also observed a decrease in pH as the fermentation time increased. However, the pH values obtained at the end of this experiment were higher than that of Ezenekwe et al. (2021) and Raji et al. (2012).

Effect of oxygen assisted fermentation on specific gravity

The specific gravity decreased as the fermentation time increased for the first six samples as shown in Figure 4. Values ranged from 1.008 to 1.004. There was no effect on specific gravity for sample seven. These results differ from the results of Ezenekwe et al. (2021), who recorded an increase in specific gravity as the fermentation period increased.

Effect of oxygen assisted fermentation on acetic acid content

Figure 5 presents variation in acetic acid production as a function of oxygen concentration during fermentation. Samples 6 and 7 had very low acetic acid content compared to the other samples. Samples 1-5 showed an increase in acetic acid value and the sample with the highest oxygen concentration had the highest acid content. This could be because there was enough oxygen available for the conversion of alcohol to acetic acid by acetic acid bacteria. Acetification is an aerobic process, and oxygen is critical to the growth of the bacteria (Mas et al., 2014). This therefore suggests that the higher the oxygen saturation, the more acetic acid is being produced during fermentation. This was in line with the results of Sossou et al. (2009), Raji et al. (2012), Ezemba et al. (2021) and Song et al. (2022). Deficiency of oxygen strictly reduces acetic acid production and can lead to cell damage, meanwhile sufficient oxygen supply maintains a reasonable energy metabolism and cell tolerance to improve acetic acid fermentation (Zheng et al., 2018). The results confirm that in order to catalyze the reaction that provides energy, acetic acid bacteria require an adequate supply of oxygen (Spinosa et al., 2015).

Effect of oxygen assisted fermentation on Antioxidant activity

All samples had higher antioxidant activity as compared to the commercial vinegar (Figure 6). Samples without oxygen had the highest antioxidant activity compared to the treated samples. These results indicate that processing alcohol to vinegar reduces the antioxidant activity of the product. These results suggest that the antioxidant activity of pineapple peel vinegar reduces when using pure oxygen. It also suggests that if the oxygen is sufficient then the antioxidant activity will be more compared to insufficient oxygen supply. The difference was not significant among the samples, but it was higher than the commercial vinegar and observed to decrease from the positive and negative controls. However, Bakir et al. (2016) concluded that

Table 3. Physicochemical properties of pineapple peel.

Variable	Before pre-treatment	After pre-treatment and neutralization	After fermentation
pH	/	7.02	5.38
TSS (^{o}brix)	6	11.5	6
SG	1.000	1.012	1.009
Glucose concentration (mg/dl)	300	580	62
TA(g/100ml)	/	/	0.6
Alcohol content (%)	/	/	7

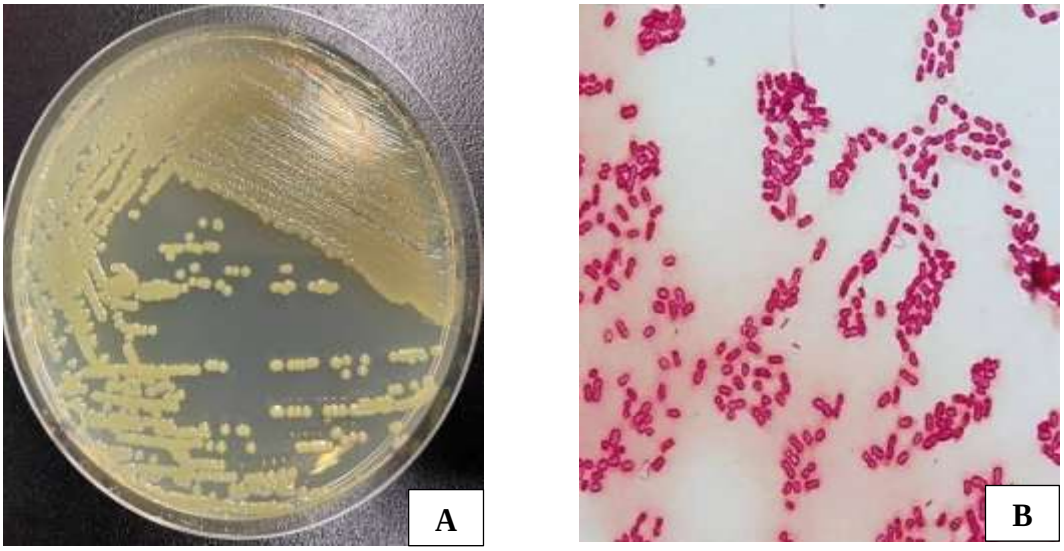

Figure 1. Acetic acid bacteria culture on a GYC medium (A) and Gram Staining (B).

Table 4. Results of bacteria identification.

Character evaluated	Results
Inoculation on GYC medium	Growth observed
Shape	Rod
Color	Whitish
Elevation	Flat
Surface	Smooth and shiny
Gram stain	Gram negative
Catalase	Positive
Oxidase	Negative
Odor	Like that of vinegar
Identity	*Acetobacter*

spectrophotometric methods indicate a strong loss of antioxidant phenolic compounds during the transition from fruit wine to fruit vinegar. The higher antioxidant activity confirms the findings of Kulkarni (2015), who observed that pineapple peel vinegar had a higher antioxidant activity compared to that of the fruit and concluded that pineapple peel vinegar can be produced in large scale and marketed for its therapeutic effects. Also, higher antioxidant capacity found in the pineapple peel vinegar can be explained by the higher

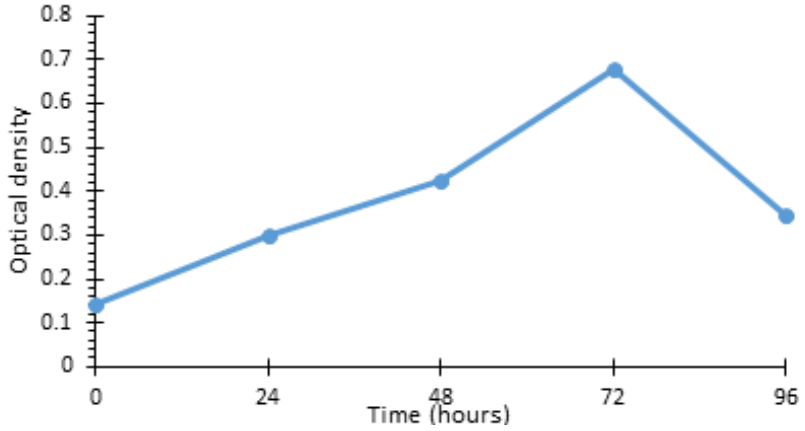

Figure 2. Evolution of optical density of isolated acetic acid bacteria.

concentration of phenolic compounds and organic acids commonly found in the fruits, which have antioxidant activity. Higher values were reported by Fonseca et al. (2018) in blueberry wine and honey for all of the antioxidant methods evaluated. However, the results of Vahos et al. (2020) revealed that alcoholic beverages had the highest antioxidant activity; after acetic fermentation, a decrease in antioxidant potential was observed in all three extractive processes evaluated, including FRAP.

Effect of process parameters on the physicochemical and antioxidant properties of vinegar

A central composite design was used to study the effect of process parameters on the production of vinegar from pineapple peels. The process variables evaluated were the oxygen concentration (A), fermentation time (B) and bacteria inoculum (C). The main responses considered were acetic acid concentration, specific gravity and antioxidant potential (FRAP). Other responses were pH, total soluble solids (TSS), temperature and density. Table 5 presents the experimental outcome of the 16 runs generated by the experimental design.

Effect of process parameters on acetic acid content

The model equation depicting the influence of process parameters on acetic acid concentration is represented by equation 2.

$$Y_{\text{Acetic acid content}} = -10.1444 + 0.0484253A + 2.04056B + 0.603834C - 0.000239605A^2 + 0.000604167AB + 0.000325AC - 0.169258B^2 - 0.009375BC - 0.0155554C^2\ldots \quad (2)$$

With R^2 of 86.22% (greater than 70%), the equation is said to be valid.

Bacteria inoculum (C) and time (B) had significant effects on the acetic acid content of the vinegar, with p-value less than 0.05 (Appendice). Doubling time had a negative effect on acetic acid content, while bacteria inoculum had a positive significant effect (Figure 7). All other factors and interactions showed no significant effect on the acetic acid content. The interaction of time and bacteria inoculum, doubling inoculum and doubling oxygen concentration had negative effects, though not significant, while interaction of time and oxygen, oxygen and bacteria inoculum, oxygen concentration had positive, non-significant effects on the acetic acid concentration.

Chalchisa and Dereje (2021) stated that bacterial inoculum and fermentation time were the most important factors affecting vinegar production.

Increasing the bacteria inoculum increases the acetic acid concentration. This was in line with the results of Saha and Banerjee (2013), who found out that increasing the bacteria quantity during banana vinegar fermentation, increases the acetic acid concentration. In their work, the highest acidity of 4.67 was observed in the sample with highest amount of bacteria inoculum. Production of acetic acid from ethanol depends on the presence of acetic acid bacteria (Song et al., 2022).

Increasing the fermentation time increases the amount of acetic acid in the sample. But doubling the time rather reduces the acetic acid content. This show that time has both a positive and negative effect on acetic acid content of vinegars. Aye (2016) found out that the acidity of the vinegar produced decreased when the fermentation period was longer, and the negative effect observed could be caused by the further oxidation of acetic acid. Also, acetic acid has toxic effects on acetic acid bacteria when the concentration becomes greater than the bacteria can tolerate (Song et al., 2022). The best results are obtained when acetic acid was being maximized. For maximum acetic acid content, the Optimum value = 4.34504, under the optimum conditions of 121.123 oxygen volume, 137 h and 18.9 ml of acetic acid bacteria.

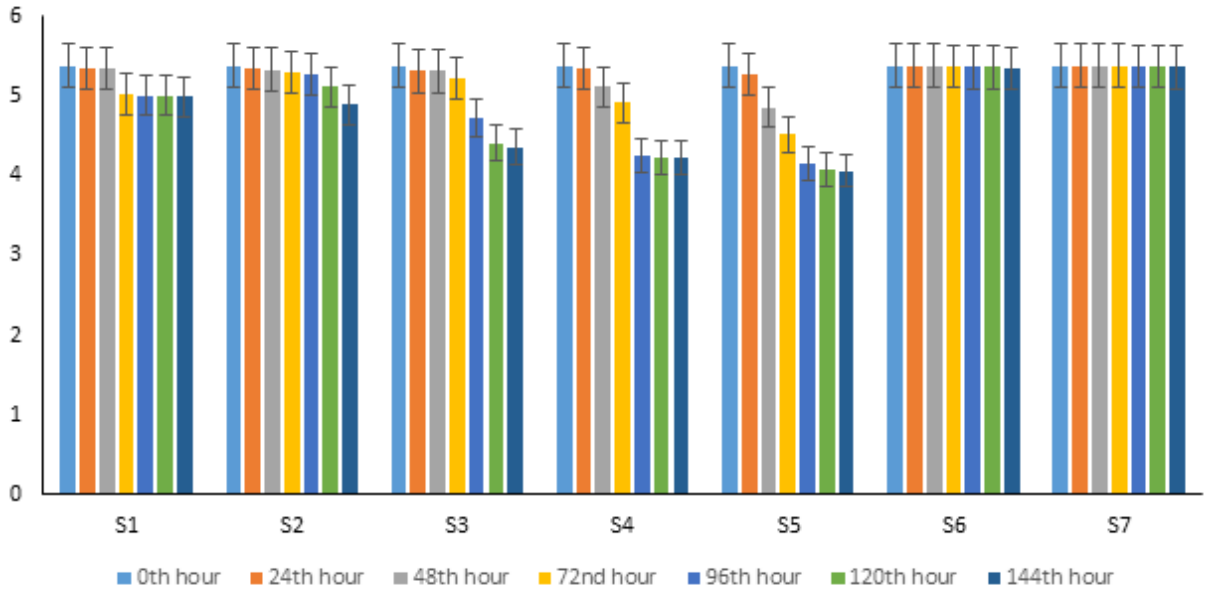

Figure 3. Effect of oxygen assisted fermentation on pH.

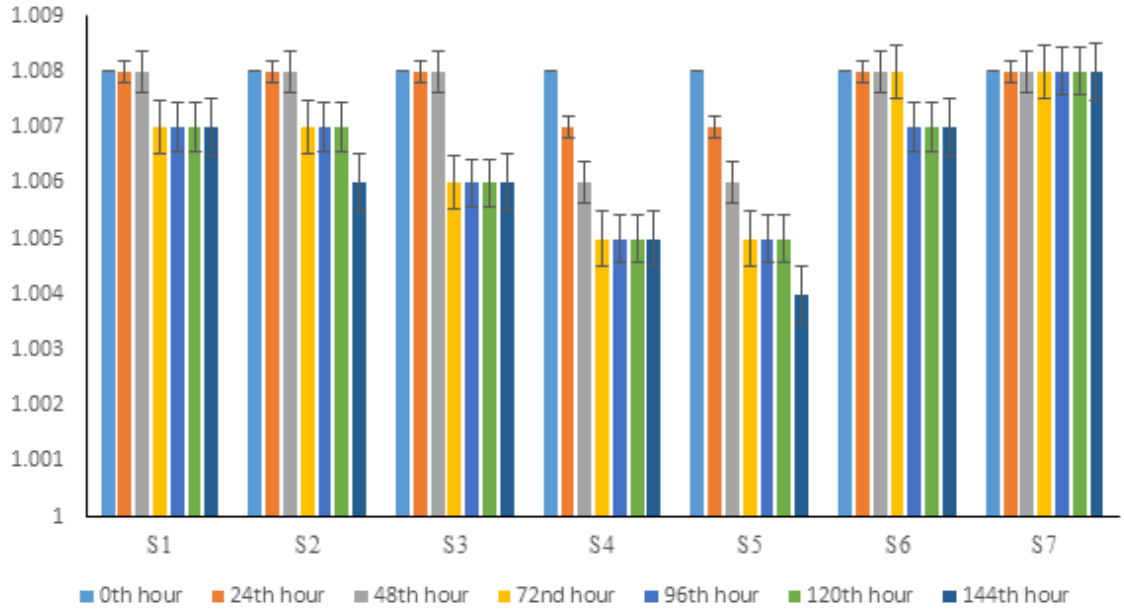

Figure 4. Effect of oxygen assisted fermentation on specific gravity.

The optimum acetic acid content is in line with the recommended value for vinegar production.

Effects of process parameters on pH of vinegar

The model equation predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on pH of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as shown in equation 3:

$$Y_{pH} = 5.96163 - 0.0378167A - 0.0907543B + 0.0592418C + 0.000186719A^2 + 0.0013125AB + 0.000175AC + 0.00179514B^2 - 0.004375BC - 0.0019048C^2 \quad (3)$$

Where pH is the response. The equation is not valid since R^2 is 58.6073, which is less than 70%.

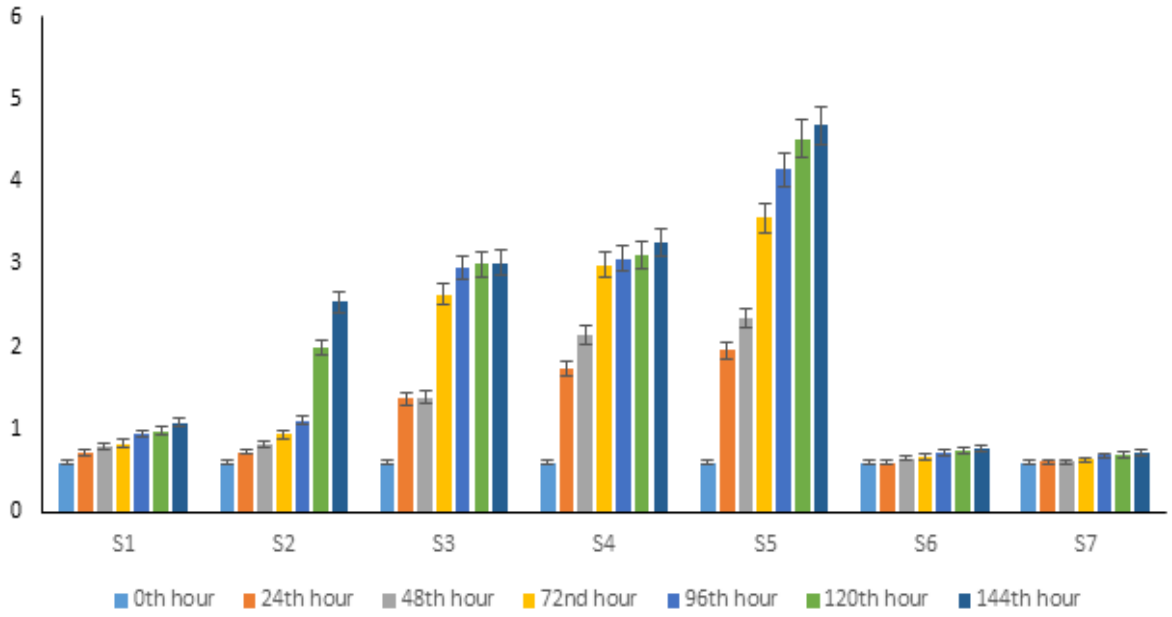

Figure 5. Effect of oxygen assisted fermentation on acetic acid content.

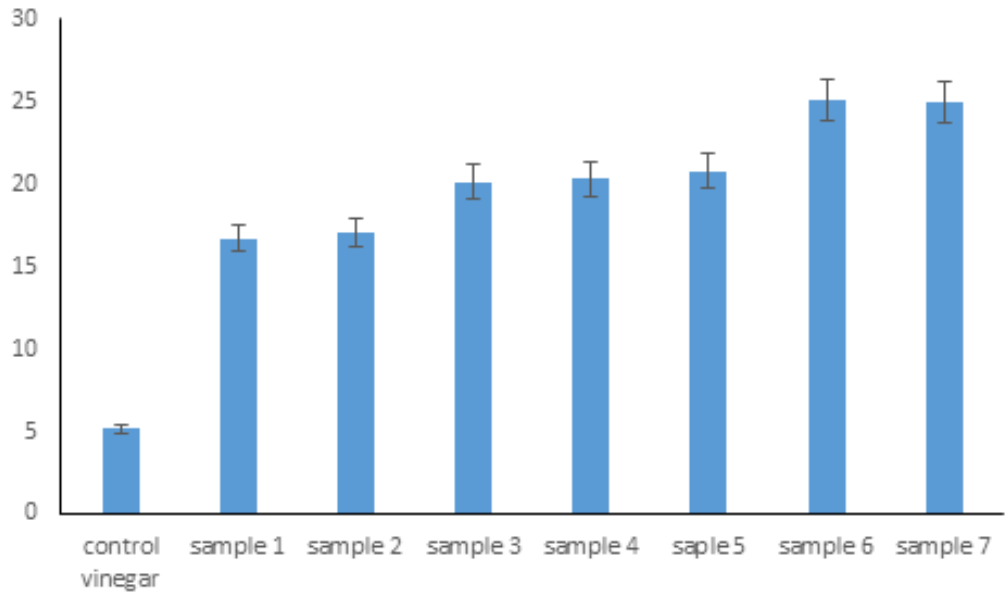

Figure 6. Effect of oxygen assisted fermentation on antioxidant activity.

No factor had a significant effect on the pH. Time, doubling bacteria inoculum, interaction of oxygen concentration and bacteria inoculum and doubling oxygen concentration had negative non-significant effects on the pH of the vinegar produced. Doubling Time had a positive non-significant effect on the pH of the sample, together with oxygen concentration, bacteria inoculum, interaction of time and oxygen concentration and interaction of time and inoculum (Figure 8). This reveals that there is a decrease in pH with increasing length of time which is in line with the report of Ezenekwe et al. *(*2021). The decrease in pH could be attributed to the fact that the alcohol present is being oxidized to acetic acid, which then lowers the pH of the sample. The effect of process parameters on pH is in line with the findings of Mizzi et al. (2022), with no significant difference between the initial and final pH values. Therefore, pH does not provide a good indication of the fermentation process.

Table 5. Effect of process parameters on the physicochemical and antioxidant activities of vinegar produced by two stage oxygen assisted fermentation of pineapple peel biomass.

		Responses					
Runs	**acetic acid concentration** (g/100ml)	**specific gravity**	**FRAP**	**pH**	**TSS (%)**	**Temperature (°C)**	**Density**
1	1.5	1.007	24.7	4.66	5.5	31.2	1.2056
2	3.34	1.006	17.03	4.55	5.7	30.5	1.2059
3	3.4	1.007	23.9	4.98	5.8	31.2	1.2073
4	1.99	1.008	14.43	4.48	6	30.1	1.2045
5	2.2	1.007	19.7	4.83	6	30.4	1.2085
6	2.99	1.006	20	4.54	5	31	1.2037
7	1.56	1.008	28.9	4.47	5.8	30.3	1.2066
8	3.24	1.007	14.9	4.83	6	31.3	1.209
9	3.97	1.007	14.9	4.48	5.2	30	1.2031
10	1.56	1.008	15.6	4.55	6	29.7	1.2072
11	3.12	1.006	18.5	4.76	6	31	1.2053
12	0.66	1.008	14.33	5.89	5	31.5	1.0057
13	4.26	1.006	14.9	4.48	5.4	31.9	1.204
14	4.52	1.007	16.6	4.69	5.8	31.7	1.2038
15	2.5	1.007	21.6	4.72	5.4	29.9	1.2049
16	2.64	1.008	18.1	4.76	6	31	1.2104

The results for each response was generated separately and fitted into the model equation CCD, then analyzed separately.

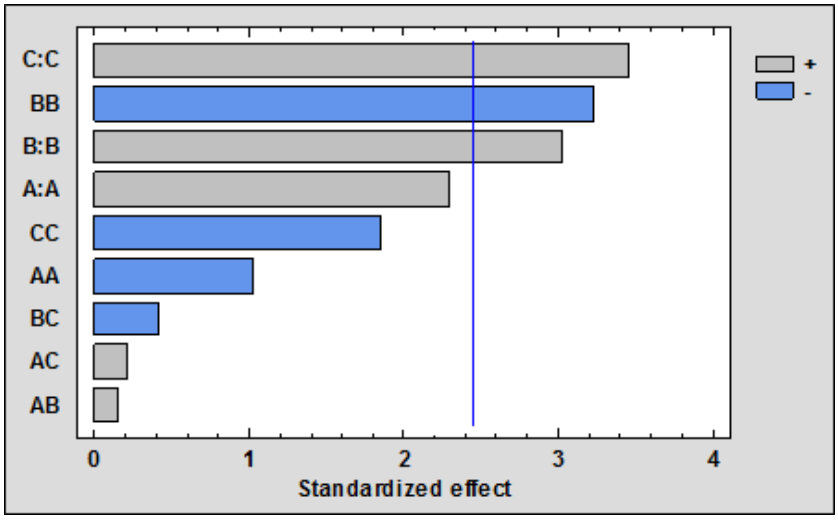

Figure 7. Pareto diagram showing the effects of process parameters on the acetic acid content of the vinegar produced.

Optimize Response for pH was 5.4 with processing conditions of 122.5 oxygen volume, 1.6 day and 14.7 ml inoculum

Effects of process parameters on TSS/Brix

The model predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on TSS/Brix of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as presented in equation 4:

$$Y_{TSS} = 7.69267 - 0.0298656A - 0.0503593B - 0.082121C + 0.000141053A^2 + 0.00104167AB - 0.00025AC - 0.00361831B2^2 + 0.00875BC + 0.000835283C^2 \quad (4)$$

Four effects have *p*-values less than 0.05, indicating that

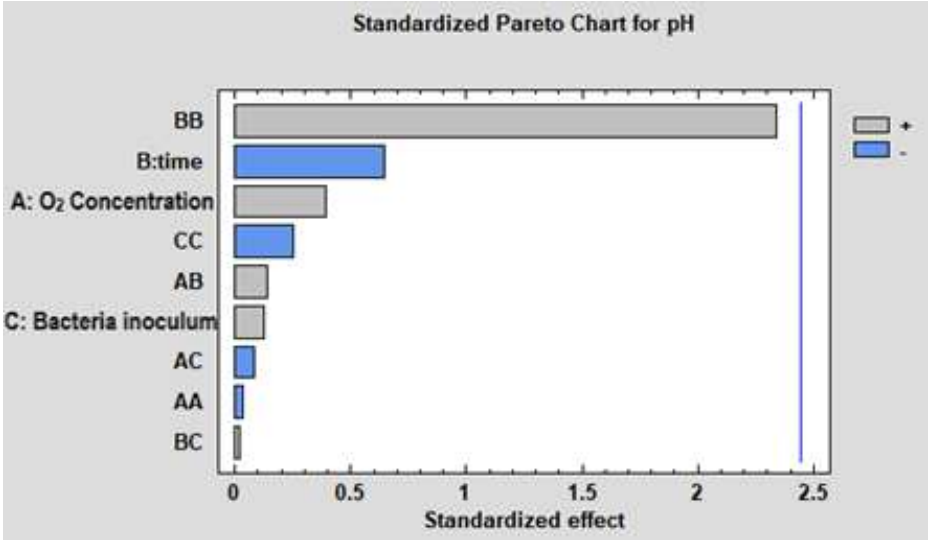

Figure 8. Pareto diagram showing the effects of process parameters on pH of vinegar.

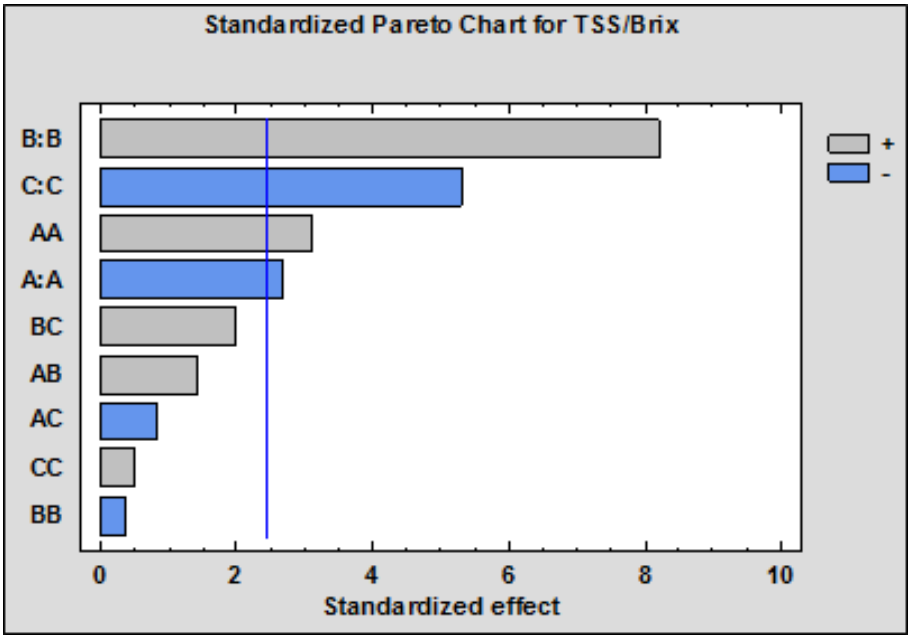

Figure 9. Pareto diagram showing effects of process parameters on TSS/Brix of vinegar.

they are significantly different at the 95.0% confidence level. Bacteria inoculum had a negative significant effect on the TSS, together with oxygen concentration, while time and doubling oxygen concentration had significant positive effects (Figure 9). The TSS values however were higher than that obtained by Akarca et al. (2020), who had mean values of 3.63±0.07 °Brix.

Bacteria inoculum had a significant negative effect on the total soluble solid of the product with a p-value of 0.0018. This indicates that increasing the amount of bacteria reduces the TSS in the vinegar. The minimum brix value obtained was 5.2% and the maximum was 6%.

Oxygen volume had a significant positive effect on the total soluble solid of the product with a *p*-value of 0.0366. This indicates that increasing the amount of oxygen will increase the TSS in the vinegar. However, doubling the oxygen volume had a significant negative effect on the TSS of the product, with a *p*-value of 0.0213. Time had a

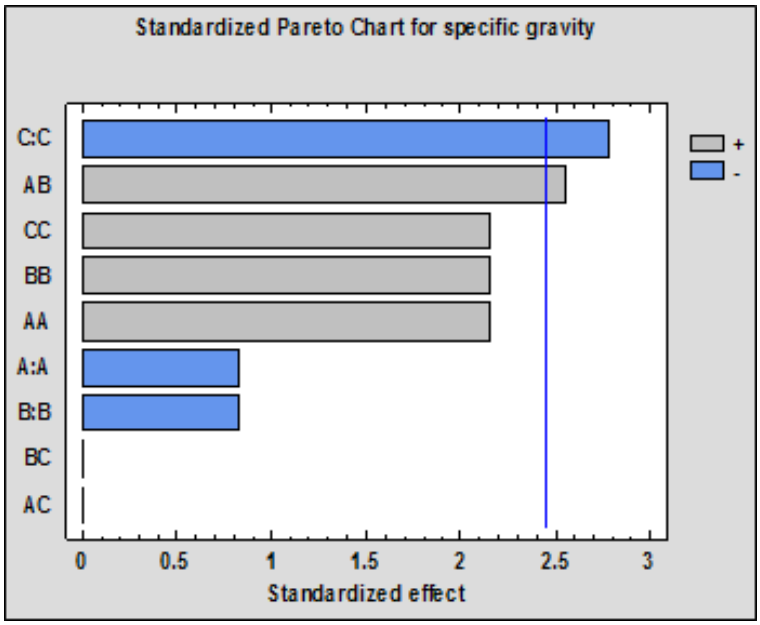

Figure 10. Effects of process parameters on specific gravity of vinegar.

significant positive effect on the TSS of the product with a *p*-value of 0.002. This indicates that increasing the time will increase the TSS in the vinegar.

Effects of process parameters on specific gravity

The model predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on specific gravity of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as shown in equation 5.

$$Y_{Specific\ gravity} = 1.02125 - 0.000124304A - 0.00179323B - 0.000554406C + 4.36292E\text{-}7A^2 + 0.00000833333AB + 0.0AC + 0.000098166B^2 + 0.0BC + 0.0000157066C^2 \quad (5)$$

The equation is valid since the R-squared = 79.4904%, greater than 70%. The significant effects of process parameters on vinegar specific gravity are illustrated by a Pareto diagram shown in Figure 10. Bacteria inoculum had a significant negative effect on the specific gravity of the vinegar produced while interaction of time and oxygen concentration showed a positive significant effect on the specific gravity of vinegar produced. Other factors and interactions had no significant effect. These results show that increasing bacteria inoculum will reduce the specific gravity of the vinegar.

Effect of Bacteria inoculum on the specific gravity of vinegar: As the amount of bacteria increases, the specific gravity reduces. Bacteria inoculum has a significant negative effect on the specific gravity of the vinegar produced with p-value of 0.0319.

Effect of time and oxygen concentration on specific gravity: As the amount of oxygen increases, together with time, the specific gravity also increases as shown in Figure 11. They have a significant positive effect with a p-value of 0.0430.

Effects of process parameters on ferric reducing antioxidant activity of vinegar

The model predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on FRAP of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as depicted by equation 6:

$$Y_{FRAP} = 86.7586 - 0.503245A - 8.22198B - 3.49431C + 0.00159595A^2 + 0.02AB + 0.008AC + 0.27954B^2 + 0.2075BC + 0.0537067C^2 \quad (6)$$

Figure 12 is a standardized pareto chart showing the influence of factors on FRAP. No significant effect was observed on the antioxidant activities of the vinegar, although the values were higher than that of commercial vinegar. However, time and bacteria inoculum had negative effects on the ferric reducing antioxidant activity of the vinegar. On the other hand, every other factor and interaction had positive effects. Results of multiple response optimization analysis revealed optimum

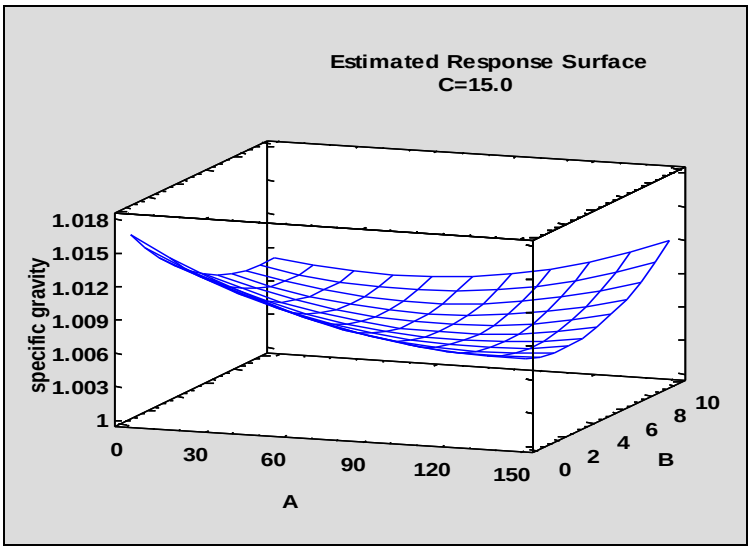

Figure 11. Effect of time and oxygen concentration on specific gravity of vinegar.

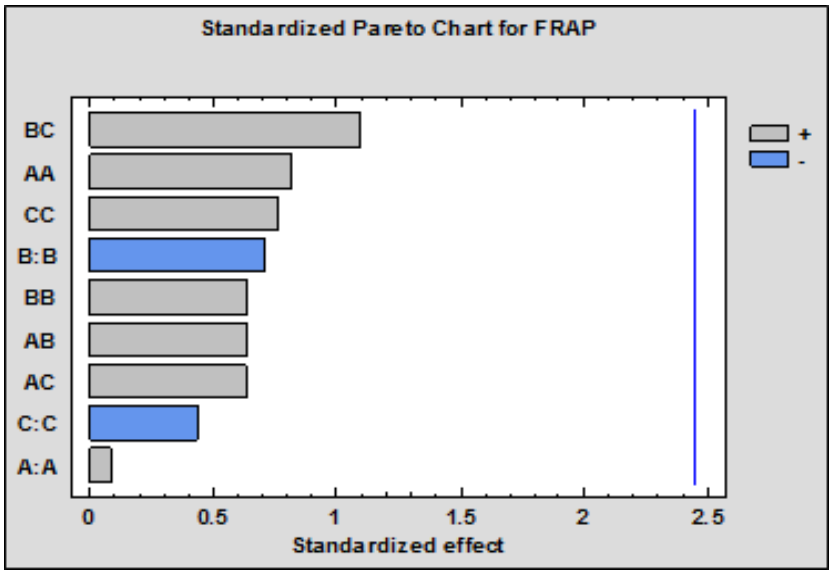

Figure 12. Pareto diagram showing the effects of process parameters on ferric reducing antioxidant activity of vinegar.

conditions of 8 days, 18.81 bacteria inoculum volume and 140 ml oxygen saturation and predicted optimum pH, TSS, temperature, acetic acid content, specific gravity and density to be 5.21, 6.0, 31.6, 3.1, 1.007 and 1.19882 respectively. The predicted optimum ferric reducing antioxidant activity was 28.97. The predicted acetic acid concentration which was the main response fell below the standard. This could be because of other factors. However, the predicted acetic acid content was closer to that of Jang et al. (2009), with a predicted acetic acid content of 3.77%.

Conclusion

The main objective of this research was to contribute to the utilization of food waste by creating vinegar through a two-stage fermentation process that involves the use of pineapple peels, a type of lignocellulose biomass. This innovative approach incorporates the assistance of oxygen to enhance the fermentation process. The findings of the study demonstrated that this two-stage oxygen assisted fermentation technique had significant impacts on key attributes of the produced vinegar,

including pH, specific gravity, and acetic acid content. Interestingly, the research indicated that the ferric reducing antioxidant activity of the produced vinegar remained unaffected by the fermentation process. While this activity did not exhibit significant changes, it's noteworthy that the values surpassed those observed in commercial vinegar products. The study also investigated the influence of various process parameters—namely, time, bacteria inoculum, and oxygen volume—on several physicochemical properties of the vinegar, such as acetic acid content, specific gravity, total soluble solids (TSS), and temperature. Notably, these parameters had varying effects on the studied properties. Importantly, the pH of the vinegar, as well as its density and antioxidant activity, were found to be unaffected by the tested parameters. Despite the potential additional cost associated with using pure oxygen, the two-stage oxygen assisted fermentation technique presents itself as a promising method for industrially producing vinegar from pineapple peels and other agro food waste. This approach offers several advantages, including its environmentally friendly nature, shorter production time and the product is free from contamination.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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