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OPTIMIZATION OF VINEGAR PRODUCTION FROM PINEAPPLE PEELS SUBSTRATE USING *SACCHAROMYCES CEREVISIAE* AND *ACETOBACTER PASTEURIANUS*

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ABSTRACT

Saccharomyces cerevisiae (yeast) and Acetobacter pasteurianus were isolated from palm wine and fermented pineapple. Vinegar was produced by anaerobic fermentation of pineapple peels for 72 hours using the Saccharomyces cerevisiae to make an alcoholic medium. The alcoholic medium was then inoculated with the Acetobacter pasteurianus for the final vinegar production. The production process was set up for optimisation, lasting four weeks. The varying parameters used for the optimisation process included the amount of pineapple (60 g, 90 g and 120 g), sugar concentration (15 g, 20 g and 25 g), temperature (30 ℃, 33 ℃, and 36 ℃) and time (between 7 and 28 days). The factor used to measure performance was the concentration of accumulated acetic acid in the vinegar solution. The treatment containing 60 g of pineapple and 25 g of sugar and fermented at 30°C for four weeks was the best-performing setup with the highest level of acetic acid of 40.2 g per litre. Statistical results revealed that the amount of pineapple peels (60 g), sugar level (25 g), and fermentation time significantly impact the concentration of acetic acid that was accumulated in the vinegar. However, the temperature difference had no significant contribution to the amount of acetic acid accumulated in the vinegar. Therefore, vinegar can be optimally

produced using 60 g of pineapple peels, 25 g of sugar, fermented at room temperature (30℃) for 4 weeks.

Keywords: Vinegar, *Saccharomyces cereviseae*, Fermentation, *Acetobacter pasteurianus*

INTRODUCTION

Vinegar is made from alcoholic and acetic acid fermentation of carbohydrates by yeast and acetic acid bacteria. Acetic acid is also called ethylic acid, ethanoic acid, methane carboxylic acid or vinegar acid. Fermentation primarily produces it (Virginia Department of Health, 2023). It is a colourless liquid with a pungent odour, used as a food acidulant, preservative, fungicide, herbicide, microbiocide and pH adjuster (Pravasi, 2014). Acetic acid causes the fragrant smell of vinegar. A laboratory procedure can obtain a higher concentration of acetic acid in vinegar. Glacial acetic acid contains small traces of water and is very corrosive (Virginia Department of Health, 2023). The concentration of acetic acid in vinegar is between 4 and 6 per cent, which is not harmful for human consumption. However, it irritates the eye, skin, mucous membrane, nose and throat at higher concentrations. Protracted exposure to concentrated acetic acid could result in pharyngitis, conjunctivitis, bronchitis and damage to teeth (Virginia Department of Health, 2023). Inhaling acetic acid fumes could cause breathing difficulty (Pravasi, 2014). Acetic acid has been helpful in the prevention of the growth of fungi. Among organic acids, acetic acid shows the highest inhibitory effect against *A. flavus* (Hassan *et al*., 2012). Vinegar lowers blood glucose levels in people with type 2 diabetes mellitus, reducing the risk of heart diseases and promoting weight loss.

Acetic acid bacteria are gram-negative obligate aerobes. They are positive for the catalase test but negative for the oxidase test. They are either ellipsoidal or rod-shaped cells. They could be in chains, pairs, or singles. They are mesophilic with an optimum temperature of 25℃ -30℃ and pH of 5.0-6.5 (Gomes *et al.*, 2018). Acetic acid bacteria belong to either of the following genera: Acetobacter, Gluconacetobacter, Gluconobacter and Komagataeibacter. The Acetobacter genus comprises three species: *A. pasteurianus, A. aceti* and *A. peroxydans* with nine subspecies. The Gluconobacter genus contains one species, G. oxydans, with four subspecies. It is classified based on the inability to oxidise lactate and acetate, presence or absence of polar flagella, ability to oxidise D-glucose to gluconate, and gluconate to 5-ketogluconate and 2-ketogluconate (Gomes et al., 2018). Acetic acid bacteria are obligate aerobes used in vinegar fermentation because of their ability to oxidise ethanol, synthesise acetic acid, and acid resistance. The challenge with its industrial usage is a low production rate due to acid stress, which is a factor that affects the acid level in vinegar production (Qiu *et al*., 2021).

Pineapple (*Ananas comosus*) is a succulent tropical fruit, covered with a protective outer layer called the peel. The pineapple peel is usually discarded as waste after consuming the pineapple fruit (Mehraj *et al*., 2024). After processing pineapple fruits, the pineapple peels, which constitute about 35% of the plant, are disposed of as waste, thus constituting environmental pollution (Roha *et al*., 2013). Pineapple peels contain significant sugars like glucose, fructose and sucrose (Mehraj *et al*., 2024). According to Agarry (2012), fresh pineapple peels contain 11.2% Cellulose, 11.52% Lignin, 6.70% Pectin, 3.88% Ash, 7.0% hemicellulose, 3.13% Protein, and 71.1% Moisture. Its glucose and fructose contents are respectively 2.18% and 2.04% (Roha *et al*., 2013). Therefore,

processing this waste for vinegar production will not only ameliorate its negative environmental impacts but will also serve as a cheap raw material for biotechnological applications because it is rich in essential nutrients like carbohydrates, potassium, calcium, dietary fibre, vitamin C, and water (Mehraj *et al*., 2024). The nutritional composition of pineapple peels makes it essential for digestion and weight management (Mehraj *et al*., 2024). It can strengthen bones because of manganese and beta carotene (Delaheart, 2021). The sugar content of the fruits used is an essential parameter in vinegar production because it directly impacts the alcohol concentration, which is subsequently converted to acetic acid. The chemical composition of the initial fruit will affect the final properties of the vinegar. (Luzón-Quintana *et al*., 2021). Optimising the production process of vinegar from pineapple peels will foster production, ultimately boosting the health of locals and the shelf life of farm produce.

MATERIALS AND METHODOLOGY

Study Area

This research was conducted at the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa. New Bussa is located 16 km away from Kainji Dam at 9°53'N 4°31'E. It is the headquarters of Borgu Emirate and Borgu Local Government Area, Niger State, with an estimated population of 24,447.

Sample Collection

Palm wine (*Elaesnsis guineensis*) was collected in a sterile container from palm wine tappers. Ripe, mature and freshly harvested pineapple fruits were purchased from the local market. The sugar was purchased from the local sellers. The materials were transported immediately to NIFFR's biology laboratory.

Isolation and identification of Yeast

Yeast was isolated from palm wine using the methods of Antia *et al.* (2018). One millilitre (1 ml) of palm wine was inoculated on a prepared Sabouraud Difco Agar (SDA) plate. The plate was incubated using the Memmert Incubator I for 72 hours at 28±2°C. Then, a pure culture of the yeast isolate was obtained by subculturing a distinct colony on an SDA plate. The plate was subsequently incubated for 48 hours, and the isolate was identified by biochemical characterisation.

Production of Vinegar

The pineapple fruits were thoroughly washed, peeled, and the pineapple peels were weighed and chopped into small pieces. Essentially, 3 g of sodium metabisulfite was mixed with the 1.1 kg pineapple peels in 2 litres of distilled water to sterilise the substrate, the mixture was allowed to sit for 24 hours for the effect of sodium metabisulfite to wade off. 5 ml of isolated yeast cells in broth was then inoculated into the medium and 7.5 g of yeasts nutrient (ammonium phosphate) was added. The pineapple peels were filtered out after 72 hours, and the broth of the isolated acetic acid

bacteria was inoculated into the alcoholic filtrate and allowed to aerobically ferment for 4 weeks (Alawlaqi and Alharbi, 2014).

Confirmation test for acetic acid in vinegar

The presence of acetic acid was determined by testing for odour. This was executed by wafting the top of a test tube containing 5 ml of vinegar to the nose to perceive the characteristic pungent smell of vinegar. A confirmation test was executed by reacting 5 mL of the vinegar solution with 0.2 g of sodium bicarbonate.

Optimisation of Vinegar Production Process

The Minitab Statistical tool was used for randomisation to obtain an unbiased experimental design. Randomised parameters using the Minitab statistical tool resulted in 27 treatments (control treatment inclusive), with 108 runs in four weeks. The concentration of acetic acid in each setup was recorded every week. Vinegar production optimisation was done by varying Pineapple peels (60g, 90g and 120g), sugar concentration (15g, 20g and 25g), temperature (30°C, 33°C and 36°C) and time (1week, 2 weeks, 3 weeks, and 4 weeks). These parameters were used for the optimisation setup. However, 250 ml of distilled water was used as the solvent for all treatments. The treatment combinations are displayed in Table 1 below.

Determination of Acetic Acid Concentration in Vinegar

The concentration of acetic acid in each setup was determined by adding 5 ml of vinegar to a 50 ml conical flask and then titrating with 0.5 mol of sodium hydroxide solution. Phenolphthalein was used as an indicator to signal the endpoint. The titration was executed, and the volume of sodium hydroxide used was recorded as soon as the medium turned pale pink (end point) (Pepin *et al*., 2020). The amount of acetic acid present in the vinegar was then calculated using the following equations;

$$\text{Mol}_{CH3COOH} = \frac{\text{Mol of NaOH} \times \text{Volume NaOH}}{\text{Volume CH3COOH}}$$

$$\text{Mass (g)} = \text{Mol}_{CH3COOH} \times \text{Molar Mass (60)}$$

Data Analysis

The study's data were subjected to one-way ANOVA, which was analysed using the SPSS version 24.0 statistical tool. The statistical results were interpreted appropriately.

RESULTS AND DISCUSSION

Production of vinegar

Vinegar was produced by fermenting pineapple into an ethanolic medium using *Saccharomyces cerevisiae.* The ethanolic medium was subsequently converted into vinegar by *Acetobacter pasteurianus*, which produced acetic acid. Plates 1 and 2 show images of the alcoholic and acetic acid fermentation stages of vinegar. The solution produced a pungent odour, which suggested the presence of acetic acid.

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Plate1: Alcoholic Fermentation Phase of Vinegar

Plate 2: Acetic Acid Fermentation Phase of Vinegar

Confirmation of acetic acid in vinegar

When wafted to the nose, the vinegar solution produced a pungent smell. The presence of colourless gas confirmed the presence of acetic acid after the solution's reaction with sodium bicarbonate.

Optimisation of vinegar

The optimisation result shows that treatment G (60 g Pineapple, 25 g sugar at 30 °C) was the best performing treatment with the highest level of acetic acid at the end of the third and fourth week, with a respective concentration of 38.4 g and 40.2 g. Treatments E (60 g of pineapple, 20 g of sugar, 33 °C), I (60 g of pineapple, 25 g of sugar, 36 °C) and G (60 g Pineapple, 25 g sugar at 30 °C) shows consistent increase in acetic acid concentration from the first week through fourth week. In contrast, the acetic acid level in other treatments fluctuated. This fluctuation could be explained by acetic acid bacteria's oscillatory growth due to acetic acid's toxic effect on the culture organism, leading to autolysis (Krusong *et al*., 2014). Treatment X (120 g of pineapple, 20 g of sugar, 36 °C) recorded the lowest level of acetic acid, with a concentration of 11.4 g at the end of the fourth week. All the low-performing treatments contain 120 g of pineapple. Except for treatments E, G, H, I, K and U, all treatments had a decrease in acetic acid concentration at the end of the fourth week. The control treatments also dropped the acetic acid concentration at the end of the fourth week. Statistical test at p=0.05, revealed that there were significant difference in the concentration of acetic acid due to the amount of pineapple; $F(2, 24) = 5.360$, $p = 0.012$, sugar concentration $F(3,90) = 24.050$, $p < .001$, and fermentation time $F(3, 104) = 25.641$, $p < 0.001$. Temperature variation, $F(2,90) = 0.299$, $p = 0.742$, had no significant impact on the concentration of acetic acid in vinegar. Table 2 summarises the statistical significance of pineapple, sugar, temperature and time on acetic acid production by AAB.

DISCUSSION

Saccharomyces cerevisiae and *A. pasteuranus* were isolated from palm wine and fermented pineapple. They were used as the culture organisms for vinegar production, which involved an alcoholic fermentation phase by *S. cerevisiae* and an acetic acid fermentation phase by *A. pasteuranus* (Krisha, 2023). The sugar supplement and the sugar component in pineapple peels were converted into alcohol by *S. cerevisiae.* The ethanolic conversion involved two steps; first, sugar was broken down into two moles of pyruvate. The pyruvate was then converted into two moles of ethanol and two moles of carbon dioxide (Yang *et al*., 2007). Saccharomyces cerevisiae is the most widely used organism for ethanol fermentation (Fernandes *et al*., 2022). The efficient yield of ethanol, rapid growth rate and high tolerance to environmental stress like low pH, low oxygen, and high concentration of ethanolic solution make *Saccharomyces cerevisiae* an efficient organism for ethanol production (Ruchala *et al*., 2019). *Acetobacter pasteurianus* plays a key role in acetic acid fermentation, which involves the oxidation of ethanol to acetic acid via the ethanol respiratory chain (Wu *et al*., 2018). *Acetobacter pasteurianus* is often applied in vinegar brewing

Table 1: Weekly Amount of Acetic Acid Concentration for Each Treatment of the Optimization Setup

Treatment	Pineapple (g)	Sugar (g)	Temperature (°C)	Week one (g)	Week two (g)	Week three (g)	Week four (g)
A	60	15	30	9.9	19.2	25.2	20.4
B	60	15	33	16.8	23.4	24.6	19.8
C	60	15	36	18.3	19.2	18	15.6
D	60	20	30	11.7	21	29.4	28.2
E	60	20	33	**18.6**	**27**	**28.8**	29.4
F	60	20	36	15.3	25.2	27.6	25.8
G	60	25	30	**9.9**	**19.8**	**38.4**	**40.2**
H	60	25	33	15.3	**30.6**	30	33.3
I	60	25	36	**12.3**	**24**	**34.8**	36
J	90	15	30	9.9	21	19.8	18
K	90	15	33	11.2	19.2	18.6	27.6
L	90	15	36	14.1	16.2	**15.3**	14.4
M	90	20	30	12	21	27	22.2
N	90	20	33	11.1	21	22.2	18
O	90	20	36	14.4	24.6	24	22.2
P	90	25	30	12	27	34.4	33.1
Q	90	25	33	11.1	21.6	31.8	27.6
R	90	25	36	14.4	25.8	30.6	24.6
S	120	15	30	15.9	17.4	17.4	15.6
T	120	15	33	**8.1**	17.4	19.2	14.4
U	120	15	36	14.7	**15.6**	15.6	28.8
V	120	20	30	14.7	18.6	22.2	16.2
W	120	20	33	10.5	24	25.2	20.4
X	120	20	36	10.2	18	**15**	11.4
Y	120	25	30	10.5	24	28.8	25.8
XX	120	25	36	13.8	24	23.4	16.2

Key: A- XX = Treatment combinations; Randomised by Minitab statistical tool

Table 2: Statistical Results of Optimisation Procedure of Vinegar Production

Source	DF	Mean Square	F Values	*P* Values	Conclusion
Pineapple	2	61.293	3.381	.038	Significant
Sugar	3	990.750	24.050	.000	Significant
Temperature	2	14.821	0.299	.742	Not significant
Fermentation Time	3	742.474	25.641	.000	Significant

Key: Degree of Freedom (**DF**)

because it produces and tolerates a high concentration of acetic acid (Li *et al*., 2023). The study of Song *et al.* (2022) revealed that a high initial saturation gradient of the fermentation medium slows down the activities of *Acetobacter pasteurianus* in vinegar production, resulting in a low acetic acid production rate. During the fermentation of alcohol to acetic acid by *Acetobacter pasteuranus,* alcohol dehydrogenase (ADH) (on cell membrane) is first attached to pyrroloquinoline quinone to oxidise ethanol into acetaldehyde, this was followed by the subsequent conversion of acetaldehyde to acetic acid by aldehyde dehydrogenase (ALDH) (Qiu *et al*., 2021). The vinegar produced from this study contained 40.2 g of acetic acid. This is within the acceptable concentration of acetic acid in vinegar. By acceptable standards, acetic acid concentration in vinegar is within 4-6% (Virginia Department of Health, 2023). The process revealed that the best optimisation setup contained 60 g of pineapple and 25 g of sugar, which was fermented at 30 °C for four weeks. It contained 40.2 g of acetic acid. This result is supported by the findings of Gomes *et al*. (2018), which reported that sugar-rich medium at the temperature range between 25 °C and 30 °C endorses the thriving of *Acetobacter* species. The fermentation time of vinegar for 4 weeks was important for the optimisation process. Chalchisa and Dereje (2021) submitted that fermentation time is vital for vinegar production. The poor performance of treatments containing 120 g of pineapple (treatment X: 120 g of pineapple, 20 g of sugar, 36 °C) was explained by the findings of Siti *et al*. (2013), Tanamool *et al*. (2020), as well as Selvanathan and Masngut (2023), who submitted that the concentration of substrate (pineapple peels) used for the production of vinegar has qualitative effect on the final vinegar product. A high concentration of fruit sugar will result in excess alcohol accumulation. Thus, suppressing the ability of acetic acid bacteria ultimately results in the low accumulation of acetic acid in the vinegar. According to Siti *et al*. (2013), pineapple waste is composed of a high concentration of simple sugars of about 2.18% glucose and 2.04% fructose (Roha *et al*., 2013), which could result in high accumulation of ethanol when in excess (Tanamool *et al*., 2020).

The optimum temperature range for AAB is between 25-30°C (Gomes *et al*., 2018). The temperature of treatment X is outside this temperature range; this may have also contributed to the lower acetic acid concentration in this setup. However, since *Acetobacter pasteurianus* can survive at a temperature range up to 45°C, temperatures ranging between 30°C, 33°C and 36°C had no significant impact on the acetic acid production ability of *Acetobacter pasteurianus* (Yamada and Yukphan, 2008).

CONCLUSION

Isolated *Saccharomyces cerevisiae* and *Acetobacter pasteurianus* were used for vinegar production, which involved the alcoholic and acetic acid fermentation stages. The production process lasted for four weeks. The optimisation of vinegar revealed that the medium containing 60 g of pineapple peels and 25 g of sugar fermented at 30 °C was the best optimisation setup for vinegar production. Findings in this study revealed that pineapple peels, sugar and fermentation time were the most critical factors for vinegar production. In addition, there is a need for a comparative analysis of the impact of different fruit peel sources and their combination on the quality of vinegar.

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