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Optimization of process factor and characterization of vinegar-like beverage production via spontaneous fermentation from pineapple peel waste

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ABSTRACT

A feasible approach to compensating for pineapple wastage is by utilizing the waste. In the present study, pineapple peels were subjected to spontaneous fermentation to produce a vinegar-like beverage. Based on the central composite design (CCD) approach, optimization of the *process factor* recorded a maximum total acid yield, $Y_{p/s}$, and total acidity of 0.49 and 3.03%, respectively. Furthermore, the beverage possessed increased tartaric, citric, ascorbic, acetic, and ferulic acids at a maximum of 1.196%. The cytotoxicity activity toward the human colorectal adenocarcinoma cell line documented a half maximal inhibitory concentration (IC_{50}) at 3.4% v/v of the beverage. This study showcased optimized vinegar-like beverage production by indigenous microorganisms (IMO) with *pineapple* peel. The beverage contained improved organic and phenolic acids contents and antioxidant potential, which could be employed as a possible human colorectal cancer cure.

1. Introduction

Spontaneous natural fermentation can be considered as the earliest type of fermentation. However the product quality will depends on the metabolism of the existing microorganisms and the raw materials invloved. On the other hand, the carry-over benefits from its natural substrate influencing the characteristics of the final products. Although spontaneous natural fermentations produce unpredictable products, the simple process has always been preferred for food preservation or to improve the taste of the raw materials, for example kimchi and sauerkraut making is to preserve cabbage while miso, and tempeh to preserve soy bean (Seesaard & Wongchoosuk, 2022). As spontaneous fermentation using a natural substrate, that including the usage of non-consumable components of solid organic leftovers from fruit

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harvest and food preparation known as agro-waste (Patel et al., 2020). Agro-wastes are primarily comprised of lignin, celluloses, and hemicelluloses and could be exploited as fermentation substrates (Gaur et al., 2022).

Affordable carbon sources have always been an essential factor in fermentation. Accordingly, studies have focused on discovering alternative carbon sources to replace food corps to avoid competition. Pineapple peels, an agro-waste, contain high carbon, nitrogen, and mineral contents. Pineapple peels also contain high amounts of polyphenolic compounds rich in antioxidants (Kumar et al., 2021), antimalarial, anti-nociceptive, and anti-inflammatory (Ajayi et al., 2022) properties. Consequently, pineapple peel is a suitable fermentation substrate candidate (Dey et al., 2021) for manufacturing animal feed concentrates, citric acid, wine, vinegar, and vinegar-like beverages (Baidhe et al., 2021).

A vinegar-like beverage contains organic and phenolic acids or polyphenols, which are procured from microbial conversions. The acids could also be obtained from substrate carry-over through anaerobic or aerobic fermentations or both (Naraian & Kumari, 2017). Pineapple core and peel are promising fermentation substrate candidates due to their high sugar contents, up to 8.92% (Ali et al., 2020). Furthermore, pineapple peels, which make up 50% of solid pineapple waste, have been identified as fiber and enzyme (bromelain at 17.3% w/w) rich sources (Ali et al., 2020).

Numerous bacteria and fungi, including Acetobacter, Gluconacetobacter (Yanti et al., 2017), *Staphylococcus aureus*, Streptococcus faecalis, Bacillus, and Clostridium species (Omorotionmwan et al., 2019), are involved during fermentation. Similar microorganisms were also reported to colonize pineapple waste. Nonetheless, the dominant microorganisms in pineapple waste vary depending on the environment, processing, and handling. In most cases, yeast, acetic and/or lactic acid bacteria establish a complicated microbiota interaction to produce fermentative products (Cagno et al., 2010). For example, yeast could produce high ethanol concentrations with desirable aromatic compounds, such as ester, benzyl alcohol, and phenols (Rodriguez et al., 2020), while acetic acid (AAB) and lactic acid (LAB) bacteria create flavor-enhancing acids and peptides that inhibit unfavorable organism growths that might be lethal if consumed (Ewuoso et al., 2020).

The present study aimed to statistically optimize the process factors of vinegar-like beverages manufacture through spontaneous fermentation by employing pineapple peel according to the response surface methodology (RSM) method. Fermented products are typically subjected to several characterization studies to determine their quality. Commonly studied attributes include physicochemical properties, antioxidant (radical scavenging activity) and antimicrobial effects, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, total phenolic and flavonoid compounds, and organic and phenolic acids contents (Mao et al., 2022; Selvanathan & Masngut, 2021). Accordingly, the present study assessed the produced beverage for its physicochemical properties and antioxidant activities. Cytotoxicity assay against the human colorectal adenocarcinoma cells (HT-29) was also performed to determine quality improvements from employing indigenous microorganisms (IMO) to ferment the peels.

2. Materials and methods

2.1. Raw material, chemicals, and reagents

Pekan Pina Sdn. Bhd., Malaysia generously supplied the pineapple fruits of the MD2 variant employed in the present study. Upon arrival, the peels were cut, washed, drained, and stored at 4 °C for a maximum of seven days. Analytical grade glucose, citric, tartaric, lactic, succinic, malic, L-ascorbic, caffeic, p-coumaric, gallic, ferulic, and dinitrosalicylic acids, sodium hydroxide, and potassium sodium tartrate were acquired from R&M Chemicals. Other chemicals and standards with 95% and above purity, such as glacial acetic and sulfuric acids and potassium dihydrogen phosphate, were purchased from Sigma-Aldrich.

2.2. Preparation of the pineapple peel substrate and fermentation setup

The fermentation in the current study was conducted with pineapple peel juice as the substrate. The juice was procured by liquidizing the pineapple peels with an electrical blender (SBL129 Singer) at a 1:1 w/v peel to sterile deionized water ratio. The juice was collected by filtering the slurry through a 20 μm pore coffee filter. The juice was then distributed in 100 mL serum bottles with a 50 mL working volume.

Anaerobic conditions were achieved by sealing each serum bottle with 20 mm straight plug rubber stoppers (Wheaton) and aluminum crimp caps after purging them with sterile nitrogen gas (N_2) for 15 min. Conversely, the mouths of the serum bottles were plugged with cotton wool and gauze for aerobic fermentation. The media in serum bottles were not sterilized to achieve spontaneous fermentation by utilizing the IMO that naturally exists on the pineapple peels. Subsequently, the serum bottles were prepared based on the combination run suggested by the response surface methodology (RSM)-central composite design

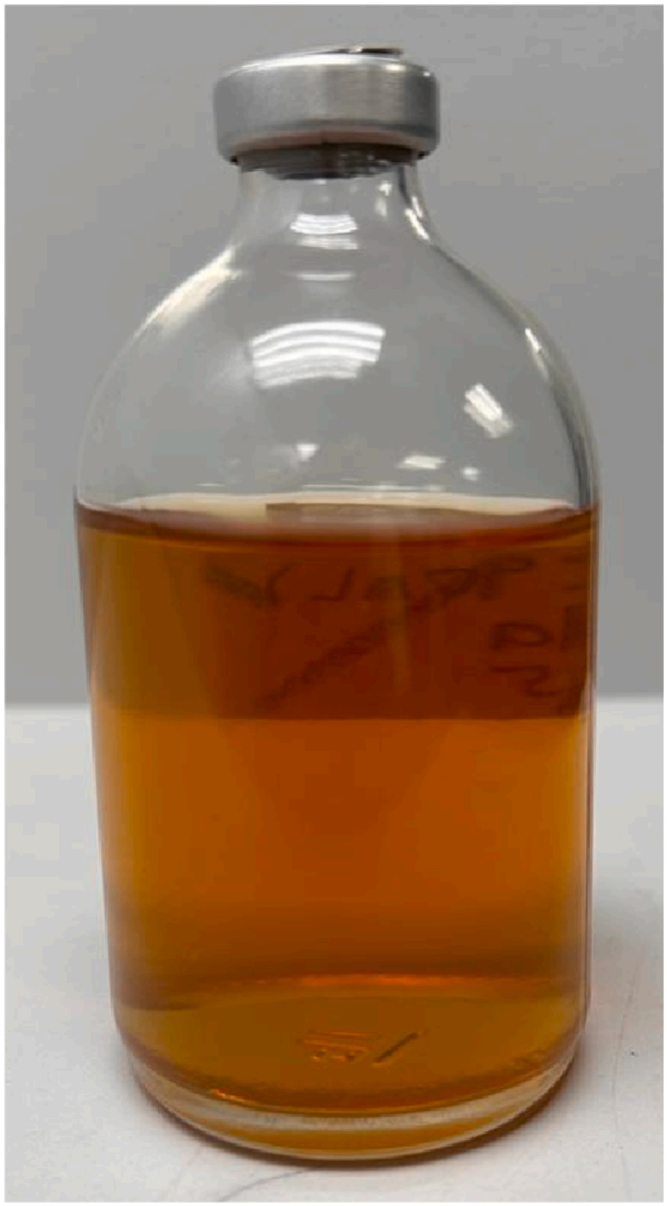

Fig. 1. The fermentation set-up for the optimization.

(CCD) approach provided by the Design Expert® Software can be seen in Fig. 1. Samples were obtained at regular intervals and centrifuged for 15 min at 8000 rpm before they were subjected to analytical procedures.

2.3. Optimization of the process factors via the RSM-CCD approach

The present study selected two significant process factors recognized in a complete factor screening evaluation by Selvanathan and Masngut (2021), which were the addition of glucose (A) and the fermentation temperature (B). A total of 13 runs were conducted at five levels (−2, −1, 0, +1, +2). The resulting acidity (Y) was the response factor. The results were analyzed by employing analysis of variant (ANOVA) with the Design Expert® (Version 8.0.6, State-Ease) software.

2.4. Quantification of acids with high-performance liquid chromatography (HPLC)

Standard organic acid solutions were procured for each acid examined in the present study, acetic, citric, tartaric, lactic, succinic, malic, and ascorbic. A Waters 2695 Alliance high-performance liquid chromatography (HPLC) system [Waters Inc., Milford, Connecticut (CT), United States of America (USA)] equipped with an ultraviolet–visible (UV–Vis) diode-array detection (DAD) was employed to quantify the acids according to the method reported by Zhang et al. (2017) with modifications.

Separation was achieved with a C18 InerSustain column of 250 mm length and 4.6 mm width. A total of 10 μL of the acids and vinegar-like beverage sample were injected at 25 °C, a 0.7 mL/min constant flow rate, and the UV–Vis spectra of the acids were analyzed at 221 nm. Potassium dihydrogen phosphate (0.02 M) was utilized as the mobile phase, which was adjusted with sulfuric acid to obtain a pH of 2.90. Finally, an HPLC grade acetonitrile and 20% acetonitrile were employed for washing.

The phenolic acids assessed in this study were quantified with the same HPLC system but analyzed at a 0.9 mL/min flow rate and a 320 nm wavelength (Ahmed et al., 2021). Aqueous formic acid (solvent A) at a 19:1 ratio and methanol (solvent B) were the mobile phases employed. The gradient elution program of the mobile phases was 75%A/25%B (0–20 min), 50%A/50%B (20–25 min), and 75%A/25%B (25–40 min). Known concentration standard solutions containing caffeic acid, p-coumaric acid, gallic acid, and ferulic acid in methanol were also prepared.

2.5. Estimation of reducing sugar

The present study conducted the dinitrosalicylic acid (DNS) evaluation according to the method reported by Teixeira et al. (2012). First, the DNS solution was prepared by dissolving 10 g of DNS in 400 mL of distilled water before warming it to 45 °C. Subsequently, 150 mL of sodium hydroxide solution (NaOH) (16 g of NaOH dissolved in 150 mL distilled water) was gradually added and stirred constantly until a clear solution was procured. Potassium sodium tartrate (300 g) was then gradually added and stirred until it completely dissolved before the solution was filtered and distilled water was added to obtain a total volume of 1 L. The vinegar-like beverage (1.5 mL) was added to 3 mL of the DNS reagent in a test tube and heated at 100 °C for 5 min. The mixture was then cooled and diluted 10 times with a citrate buffer. Finally, the reducing sugar contents of the samples were analyzed with a UV–Vis spectrophotometer (Genesys 50, ThermoFisher Scientific) at 540 nm with glucose as the standard.

2.6. Estimation of total acidity

The total acidities of the products obtained in this study were estimated via titration (Raji et al., 2012). The total acidity percentage was determined with 1.0 mL of the beverage sample, a few drops of phenolphthalein, and 0.1 M NaOH as the neutralizer. The titration procedure was performed in triplicates.

2.7. Quantification of the cells by colony-forming unit

The current study employed a mechanical vortex to homogenize the samples procured. Subsequently, 1 mL of the sample was added to 9 mL of phosphate-buffered saline (PBS) to create a serial dilution of up to 10^{-5}. A total of 10 μL of each dilution was evenly spread on nutrient agars in duplicates with a disposable spreader before being incubated overnight at 37 °C. The number of colonies on each agar was counted, and only values between 30 and 300 were considered.

2.8. The MTT cell viability assay

The HT-29 cancerous cell line was chosen to estimate the cytotoxic effect of the beverage samples procured in the present study. The vitality of the HT-29 cells was assessed through the MTT test (Mosmann, 1983). Various concentrations (0.31–10% v/v) of HT-29 cells were seeded in a 96-well plate. The well plate was then incubated at 37 °C for 12 h or until 70–80% confluency was achieved. Subsequently, 5 mg/mL of the MTT solution was added to the samples and incubated under the same conditions for 4 h. The DMSO was then added to each well. The stabilized amount of purple formazan formed from the reduction of MTT was observed with a spectrophotometer at 570 nm. Cell viability percentages were determined from the absorbance readings, and the cytotoxicity effects of the beverage samples against the HT-29 cells were recorded as half maximal inhibitory concentration (IC_{50}).

3. Results and discussion

3.1. Optimization of process factor with RSM-CCD

Considering two significant process factors previously reported by Selvanathan and Masngut (2020), five-level CCD was conducted to optimize these factors. The setup produced a total of 13 runs of a randomized design matrix. The results are summarized in Table 1.

The present study employed ANOVA to statistically interpret the data obtained (see Table 2). The ability of the regression model equations to identify deviations in the output or responses was indicated by the lower *p*-value (0.0007) and the accompanying large *F*-value (33.03) (Shivamathi et al., 2022). The R^2 (0.9788) and adjusted R_a^2 (0.9492) demonstrated a strong correlation between the independent process variables and responses. Furthermore, the low coefficient of variance indicated good reliability and high precision in the experiments conducted. A significantly low *p*-value (Shivamathi et al., 2022) denoted that the

Table 1
The results of process factor optimization via the CCD approach.

Standard order	A: Temperature (°C)	B: Glucose addition (%)	Acid production (%)		Residual (%)
			Predicted	Experimental	
1	27	7	3.01	3.093 ± 0.17	0.083
2	29	7	1.79	1.621 ± 0.04	−0.169
3	27	9	2.59	2.502 ± 0.20	−0.088
4	27	5	2,77	2.823 ± 0.25	0.053
5	27	7	3.01	3.033 ± 0.19	0.023
6	26	8	2.69	2.642 ± 0.08	−0.048
7	27	7	3.01	2.822 ± 0.14	−0.188
8	28	6	2.73	2.842 ± 0.08	0.112
9	26	6	2.62	2.432 ± 0.04	−0.188
10	25	7	1.88	2.012 ± 008	0.132
11	27	7	3.01	3.033 ± 0.17	0.023
12	27	7	3.01	3.003 ± 0.17	0.023
13	28	8	2.48	2.732 ± 0.08	0.252

All the data have been triplicated.

Table 2
The ANOVA of acid production in the pineapple peels fermented by IMO.

Source	Sum of squares	Degree of freedom	Mean of squares	*p*-value	
Model	2.20	5	0.310	0.0007	Significant
A	0.076	1	0.076	0.0367	
B	0.051	1	0.051	0.0679	
AB	0.026	1	0.026	0.1617	
A^2	1.980	1	1.980	< 0.0001	
B^2	0.160	1	0.160	0.0097	
Residual	0.048	7	$9.524 \times 10^{-0.003}$		
Lack of fit	$5.402 \times 10^{-0.003}$	3	$5.402 \times 10^{-0.003}$	0.5139	Not significant
Pure error	0.042	4	0.011		
R^2	0.9788				
R_a^2	0.9492				
R_p^2	0.6936				

results were well-fitted with the quadratic second-order polynomial model equation (Equation (1)).

$$Y = 3.01 - 0.023A - 0.045B - 0.080AB - 0.29A^2 - 0.083B^2 \quad \text{(Eq. 1)}$$

3.1.1. Interaction of process factors

Fig. 2 illustrates the three-dimensional (3D) response surface that demonstrates the interaction of factors considered in the optimization assessment towards the total acidity of the vinegar-like beverage produced in this study. The fermentation temperatures employed in the current study varied between 25 and 29 °C, which resulted in a maximum acidity of 3.03% attained at 27 °C. Sussou et al. (2009) and Roda et al. (2017) reported that the optimum temperature for the microorganisms involved in vinegar production, including yeast and Acetobacter sp., was within 25–30 °C. Moreover, Ho et al. (2017) and Li et al. (2015) found that the best temperature range to encourage yeast and AAB growths was between 20 and 30 °C.

In AAB, ethanol oxidation to acetaldehyde is catalyzed by alcohol dehydrogenase (ADH) before being further oxidized to acetic acid by aldehyde dehydrogenase (ALDH). Although the optimum temperature for ADH and ALDH was recorded within the 30–40 °C range (Neto et al., 2011), fermentations at 30 °C allowed yeast and AAB to work together, enabling simultaneous alcoholic and acetous fermentations. Consequently, a similar fermentation strategy was applied in this study. Furthermore, fermentation temperature fluctuations would affect the product (Ghosh et al., 2012), where total acidity decreased from 6.8 to 0.4% when the temperature diverted from 30 °C.

Additional sugar, 5–9% glucose, was added to the pineapple peel substrate employed in the present study and 7% glucose was identified as the optimum level (see Fig. 2). It is widely known that numerous species readily metabolize glucose as a carbon source. For instance, Raji et al. (2012) reported that adding 2.5% glucose in thin strips of pineapple peel substrate produced vinegar with 4.77% acidity. In another study, Ghosh et al. (2014) added 15% glucose to produce palm vinegar at 4.6% acidity. Nevertheless, when glucose addition was diverted from 15%, the vinegar acidity was reduced.

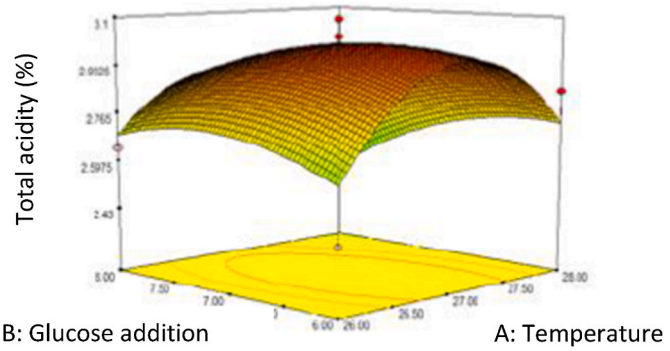

Fig. 2. The 3D response surface plots of the interactions of the studied factors and the total acidity of the vinegar-like beverage samples.

Based on Fig. 2, the total acidity in the vinegar-like beverage procured in the current study improved as the amount of glucose was increased up to 7%, depending on the fermentation conditions. Nonetheless, the acid concentration declined as the added glucose level was elevated further. In fermentations involving high initial glucose concentrations, 36% of the glucose was left in the substrate. Moreover, decreased product concentration was eminent in fermentations with high initial glucose concentrations. The observations might be due to the adverse effects of the acid and other toxic by-products accumulated during early fermentation (Bulut et al., 2014). Nevertheless, the product inhibition could be partially alleviated by further enriching the medium with necessary nutrients (Bulut et al., 2004) or in-situ product removal (Dafoe & Daugulis, 2014).

3.1.2. Validation of the optimum process factor for high acid production

The RSM-CCD employed in the current study predicted a 3.02% acidity if the suggested process factor conditions of 27 °C fermentation temperature and 7% glucose addition were met. The experimental results recorded 3.03% acidity and a 0.33% error. The findings were also compared to the data from previous studies (see Table 3).

Product acidity in the current study was lower than the level set by the Food Drug Administration (FDA), which stated that vinegar should comprise over 4% acetic acid. The present study obtained the products via spontaneous fermentation with simultaneous ethanol and acids production, which was a novelty. Consequently, the products procured in this study were categorized as vinegar-like beverages.

Employing IMO in acid fermentations has been documented to produce superior quality products compared to utilizing single-strain microbial. Liu et al. (2019) reported that a *Candida tropicalis* and yeast co-culture produced a product with enhanced oxalic, tartaric, citric, and succinic acids than the *Candida tropicalis* single-strain culture. Furthermore, umami and sweet-free amino acid levels in the co-culture were 53% higher than in the single-strain culture. The total esters, alcohols, and phenolics were also significantly increased in the mixed strain by 27.3, 75.45, and 9.23 mg/L, respectively (Liu et al., 2019).

In another study, Chen et al. (2017) demonstrated that a *Saccharomyces cerevisiae* and Lactobacillus Plantarum co-culture produced a citrus vinegar with 40% higher antioxidant activity than a Saccharomyces cerevisiae-*only culture*. Yeast, AAB, LAB, and mould populations notably affected the physicochemical properties of the products procured due to the metabolites secreted. Secondary metabolites play a pivotal role in the final product quality as they could produce broad flavour and aroma components, pigments, and even compounds with antibiotic properties (Li et al., 2015). Moreover, incorporating yeast would induce alcohol production, which might aid in higher acid production.

3.2. Product characteristics

Table 4 lists the organic and phenolic acids produced in the current study, revealing that the fermentation by IMO (spontaneous fermentation) increased the organic acids contents, such as tartaric and citric acids. The decreased malic acid level indicated its possible usage as a carbon source by the microorganisms. The phenomenon was supported by a previous study, where LAB (Gaur et al., 2022), *Saccharomyces Cerevisiae*, *Schizosaccharomyces pombe*, and *Zygosaccharomyces bailii* (Ferreira & Mendes-Faia, 2020) were observed to consume malic acid as a carbon source.

The compositions of vinegar-like beverages depend on the interactions between the IMO involved during fermentation. Organic acids typically affect the flavors of vinegar-like beverages. Changes in organic

Table 3
Comparisons of the acidity of fermented products.

Type and condition of substrate	Process factor studied	Fermentation condition	Microbial diversity	Acidity (%)	Reference
Pineapple peel juice	27 °C 5 days fermentation 7% glucose Initial pH of 4	Simultaneous fermentation (aerobic)	IMO	3.03	Current study
Thin strips of pineapple peels	25–28 °C 11 days fermentation 2.5% glucose addition 0.375% yeast addition	Anaerobic followed by aerobic with aeration	Yeast and *Acetobacter* (present by chance)	4.77	Raji et al. (2012)
Pineapple peel juice	30 °C 27–29 days fermentation	Anaerobic followed by aerobic	Yeast and *Acetobacter* strain isolated from pineapple wine	5.30	Sussou et al. (2009)
Pineapple peel and core juice	25–32 °C 37–40 days fermentation 0.025% yeast addition	Anaerobic followed by aerobic	Yeast and *Acetobacter acetii*	5.00	Roda et al. (2017)
Palm juice	30 °C 12 fermentation days 0.03% yeast addition 15% glucose addition	Anaerobic followed by aerobic with shaking at 150 rpm	*Saccharomyces cerevisiae* and *Acetobacter acetii*	6.81	Ghosh et al. (2012)
Glutinous rice (Zhenjiang aromatic)	40–46 °C 25–30 fermentation days	Aerobic solid-state fermentation	IMO	6.00	Xu et al. (2011)

Table 4
The HPLC-DAD analysis of the organic acids contents of the pineapple juice peel and vinegar-like beverage.

Acids		Pineapple peel juice	Vinegar-like beverage	Difference in percentage [positive (+) increased, negative (−) decreased) (%)
Organic	Acetic acid	N/A	1.196 ± 0.05%	100
	Lactic acid	N/A	N/A	0
	Citric acid	0.260%	0.270 ± 0.70%	3.77
	Tartaric acid	0.053%	0.156 ± 0.10%	98.56
	Succinic acid	N/A	N/A	0
	Malic acid	0.037%	0.03 1 ± 0.04%	−17.65
Phenolic	Gallic acid	135.450 μg/mL	94.370 ± 0.05 μg/mL	−35.75
	Ferulic acid	N/A	0.141 ± 0.90 mg/mL	100
	Ascorbic acid	N/A	0.022 ± 0.10%	100
	Caffeic acid	0.059 mg/mL	0.028 ± 0.08 mg/mL	−71.26
	p-coumaric acid	0.026 mg/mL	0.019 ± 0.20 mg/mL	−31.11

(*Note:* N/A = Not Available).
All the data have been triplicated.

acid contents post-fermentation are primarily due to the metabolic pathways of the microorganisms through the tricarboxylic acid (TCA) cycle, fatty acid metabolism, and some chemical reactions (Liu et al., 2021).

Vinegar comprises various acids, with acetic acid content being the highest, which contributes to its strong, pungent, and sour taste. Nevertheless, adding organic acids, including succinic, malic, citric, and lactic acids could lessen the sting from acetic acid, producing a richer and mellower-flavored fermented condiment (Xu et al., 2022). Moreover, organic acids possess various physiological functions and health benefits. For example, citric and succinic acids are crucial TCA cycle substrates with antibacterial, anti-inflammatory, and antioxidant properties (Xu et al., 2022). Furthermore, tartaric acid is widely utilized in the food, pharmaceutical, and textile industries (Liu et al., 2021).

Fermentation by IMO enhanced the phenolic acid contents in the vinegar-like beverage produced in the present study. Nonetheless, the significant decrease in caffeic, p-coumaric, and gallic acid components might be attributed to degradation and hydrolysis due to their heat and photosensitive properties (Adebo & Medina-Meza, 2020). The anti-allergy, -atherogenic, and -inflammatory, antibacterial, antioxidant, anticarcinogenic, and vasodilatory characteristics of phenolic acids are well documented (Ciniviz & Yildiz, 2020). Furthermore, the acids are natural combatant sources of cancer, cardiovascular illnesses, diabetes, and skin diseases (Ciniviz & Yildiz, 2020) and exhibited therapeutic effects on the cardiovascular, metabolic, cognitive, and gastrointestinal systems (Kahkeshani et al., 2019).

Three physicochemical properties analyses have been carried out: pH, concentration of reducing sugar, and ethanol. The pH values of pineapple peel vinegar-like beverage is pH 3.16 as shown in Table 5. Kim et al. (2012) reported similar findings from various pomegranate fruits, blackberry, blueberry, mulberry, cactus, red ginseng, and cherry with a pH between 2.81 and 3.20 and acid content up to 2.41%. It can be observed that there was a variety of pH ranges for vinegar-like beverage, and it is inversely proportional to its total acid content. This is because the lower acidity means lower hydrogen ions responsible for higher acidity but higher in hydroxide ions, thus increasing the pH value. Many hydroxide ions are essential and indicate a solution with a higher pH value and lower acidity (Avissar et al., 2013). While, the ethanol

Table 5
The physicochemical properties of pineapple peel vinegar-like beverage.

Physicochemical properties	Vinegar-like beverage
pH	3.16 ± 0.09
Reducing sugar (%)	3.18 ± 0.50%
Ethanol (%)	1.03 ± 0.15%

All the data have been triplicated.

concentration of the pineapple vinegar-like beverage is 1.03 ± 0.15%. Ethanol was an intermediate product during vinegar-like beverage production before the respected bacteria oxidized it into acids. Residual ethanol was unavoidable in vinegar-like beverage production as the conversion during acetification was around 70% (Patel & Pandya, 2015; Roda et al., 2017). The reducing sugar content was 3.18 ± 0.50% which is quite high while comparing to Kong, Kim, Jeong, Kim, and Yeo (2022) which was 0.37 ± 0.01%. Based on the result, indigenous microorganism on pineapple peel was not as efficient as pure strain to utilize the sugar.

The in-vitro cytotoxicity analysis of the vinegar-like beverage procured in the current study was performed with the MTT assay. The treated HT-29 cells exhibited a half maximal inhibitory concentration (IC_{50}) starting at the 3.4% v/v of the product (Fig. 3) that improved up to the 10% v/v following a 24-h exposure. Mohamad et al. (2019) reported that mouse mammary gland cells recorded an IC_{50} value of 0.025% after 48 h incubation in pineapple vinegar. Nascimento et al. (2022) investigated the cytotoxicity effects of bromelain in pineapples on HT-29 cells and documented an IC_{50} of 0.1% post-24, -48, and −72 h of incubation. Both data recorded relatively low cytotoxicity effects compared to the findings in the present study, revealing the anticancer properties of the vinegar-like beverage at a higher concentration.

Pineapples are the third most produced fruit after citruses and bananas (Wali, 2019). The fruit offers numerous benefits, including anti-inflammatory, muscle relaxant, and digestive aid, and it has been reported to prevent tumour growth. The anticancer effects might be associated with the antioxidant properties of the various polyphenolic components in pineapples. Nevertheless, information on the anticancer attributes of vinegar-like beverages produced by IMO is limited.

4. Conclusion

The present study explored the prospect of producing a vinegar-like beverage via spontaneous fermentation of pineapple peel substrate by IMO. The findings demonstrated that temperature and glucose addition resulted in profound effects on the acidity of the product obtained. Product characteristic assessment demonstrated increased tartaric, citric, ascorbic, acetic, and ferulic acid contents. The results provided a better insight into the utilization of the vinegar-like beverage produced from pineapple peel substrate with IMO in cancer therapy to treat colon cancer. Furthermore, the outcome verified the hypothesis that the raw materials resulted in the diverse characteristics of the products depending on the carry-over benefits from the substrate and the microorganism strain employed.

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CRediT authorship contribution statement

Yashini Selvanathan: Data curation, Methodology, Validation, Formal analysis, Analysis, Investigation, Writing – original draft. **Nasratun Masngut:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

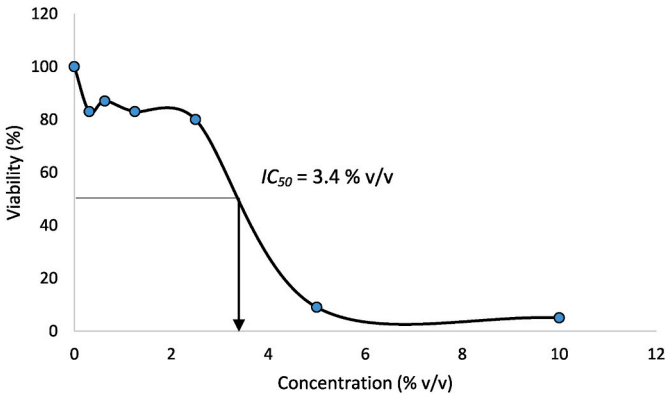

Fig. 3. Viability of the HT-29 cells at various concentrations of vinegar-like beverage produced with IMO.

Data availability

Data will be made available on request.

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