

Exploring alcoholic and acetic fermentations of Ceri Terengganu (*Lepisanthes fruticosa*): physicochemical and bioactive compound changes

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Abstract

Ceri Terengganu (*Lepisanthes fruticosa*), an underutilized exotic fruit native to Malaysia, has been overlooked due to limited cultivation and low economic value. This study aimed to highlight the potential of Ceri Terengganu by exploring its alcoholic fermentation using *Saccharomyces cerevisiae* and its acetic fermentation using *Acetobacter aceti*. The feasibility of using Ceri Terengganu as a substrate in both fermentations was examined, along with the physicochemical, antioxidant activity, and bioactive compound changes during the fermentation process. During alcoholic fermentation, yeast counts increased in the early stage and stabilized by day 3–5, while pH and total soluble solids progressively declined. The highest ethanol yield (6.41%) was recorded on day 8 with 15% sucrose supplementation. Subsequent acetic fermentation with *A. aceti* led to an increase in acetic acid concentration from 0.62% to 3.43%, accompanied by a decrease in ethanol, pH, and total soluble solids. Notably, acetic fermentation enhanced the total phenolic content of Ceri Terengganu. While the fruit itself is known for its high antioxidant activity, no significant changes in antioxidant activity were observed. However, acetic fermentation improved the flavonoids and phenolic acids content, with epigallocatechin (65.57±4.1 µg/mL) and protocatechuic acid (12.75±3.2 µg/mL) being the most abundant, respectively. The acetic fermentation product exhibited high levels of acetic acid (56790.56±56.8 µg/mL) and citric acid (7662.25±64.56 µg/mL). In conclusion, Ceri Terengganu shows promise as a substrate for alcoholic and acetic fermentations, resulting in fermented products enriched with beneficial bioactive compounds. This research sheds light on the potential value and health benefits of this underutilized Malaysian tropical fruit, contributing to its wider recognition and application in the food industry.

1. Introduction

Fermentation is a time-honoured process that has been widely utilized in the production of various food and beverage products. It involves the transformation of natural ingredients through microbial action, leading to substantial changes in flavour, aroma, texture, and nutritional composition. In recent years, there has been a growing interest in fermenting fruits and plants to not only improve their sensory attributes but also unlock their potential health-promoting benefits through the production of bioactive compounds (Arora *et al.*, 2023; Chan *et al.*, 2023)

Ceri Terengganu (*Lepisanthes fruticosa*), an

Indigenous tropical fruit native to Malaysia, has gained recognition for its exceptional nutritional profile and diverse array of bioactive compounds. This fruit contains high levels of phenolics, flavonoids, and antioxidants, which have been linked to various health benefits, such as reducing the risk of chronic diseases (Tarmizi *et al.*, 2022). However, little is known about how alcoholic and acetic fermentations impact the physicochemical properties and bioactive compound transformations of Ceri Terengganu. Although recent research has shed light on the nutritional and health-promoting aspects of Ceri Terengganu (Salahuddin *et al.*, 2017), a significant gap still exists in understanding how alcoholic and acetic fermentations affect the fruit's physicochemical

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properties and the transformations of bioactive compounds.

Alcoholic fermentation, involving the conversion of sugars into alcohol and carbon dioxide, is a widely employed process in the production of alcoholic beverages. *Saccharomyces cerevisiae*, a well-known yeast strain, is commonly used in alcoholic fermentation due to its ability to efficiently convert sugars into ethanol. Acetic fermentation, on the other hand, is the conversion of ethanol into acetic acid by acetic acid bacteria. *Acetobacter aceti*, a prominent bacterium, is often utilized in vinegar production to produce acetic acid, contributing to the characteristic tangy flavour and aroma of vinegar.

Understanding the physicochemical changes that occur during the alcoholic and acetic fermentations of Ceri Terengganu is essential for assessing the overall product quality and nutritional attributes. Parameters such as pH and total soluble solid content play crucial roles in determining product characteristics, such as flavour, texture, and shelf stability. Close monitoring of these parameters throughout the fermentation process is vital for proper process control and the production of high-quality fermented products.

In addition to the physicochemical changes, investigating the transformations of bioactive compounds during fermentation is essential in determining the potential health benefits associated with consuming fermented Ceri Terengganu products. The bioactive compounds found in Ceri Terengganu, including phenolics, flavonoids, and antioxidants, possess remarkable bioactivity, such as antioxidant, anti-inflammatory, and antimicrobial properties (Looi *et al.*, 2020; Tarmizi *et al.*, 2022). Understanding how these compounds change during alcoholic and acetic fermentations will provide valuable insights into the potential health benefits of consuming fermented Ceri Terengganu products.

Therefore, the objective of this study is to explore the alcoholic and acetic fermentations of Ceri Terengganu using *S. cerevisiae* and *A. aceti*, respectively, and investigate the physicochemical changes and transformations of bioactive compounds that occur during the fermentations. This research aimed to fill the knowledge gap regarding the impact of fermentation on the quality and potential health benefits of Ceri Terengganu products.

2. Materials and methods

2.1 Strains and cultures

Saccharomyces cerevisiae was used to produce

alcohol from glucose in alcoholic fermentation. *Saccharomyces cerevisiae* was inoculated on Potato-Dextrose (PD) agar and incubated at 30°C for 48 hrs. It was then inoculated into PD broth, incubated for 24 hrs at 30°C, and used as a seed suspension. *A. aceti* was used in acetic fermentation to produce acetic acid from ethanol. Approximately 10% of *A. aceti* from glycerol stock was inoculated on Alcohol Broth Medium (ABM) and incubated at 30°C for five days on a rotary shaker (150 rpm) and used as a seed suspension. The composition of the ABM medium was 5% yeast extract, 5% glucose, and 2% alcohol.

2.2 Ceri Terengganu sample processing

Ceri Terengganu fruit was obtained from a plantation in MARDI Station Sintok, Kedah. In the sample preparation, the fruits were thoroughly washed; cut into small sizes and the seeds were removed. They were then blended with distilled water using an industrial blender (ratio 1:2) and were filtered to get the clear juice of Ceri Terengganu. To standardize the initial concentration, the juice was adjusted to a minimum of 2.1% Brix by diluting it with distilled water. The juice was kept in a sterile container and stored in a freezer until further use.

2.3 Alcoholic fermentation

Alcoholic fermentation of Ceri Terengganu juice was carried out in 1L conical flask with a working volume of 500 mL. Different levels of sucrose concentrations were used as the supplementation (0%, 3%, 5%, 7%, 10%, 15%) and the experiments were performed in triplicates. Prior to the inoculation, the samples were pasteurized at 90°C for 20 mins. Then, all samples were inoculated with 3% (1×10^6 CFU/mL) of *S. cerevisiae*, incubated at 30°C, in static aerobic condition for 10 days and samplings were done on a daily basis. Unfermented Ceri Terengganu was used as a control.

2.4 Acetic fermentation

After the completion of alcoholic fermentation, only the sample with the highest content of ethanol was selected to be used in acetic fermentation. *Acetobacter aceti* (30% v/v) was inoculated as the starter, and the broth was incubated at 30°C under static conditions for 18 days. Fermentation was carried out in a 500 mL flask with a working volume of 250 mL. Samples of the fermenting broth were taken daily for analysis. All experiments were carried out in triplicate and the results were reported as the mean values.

2.5 Determination of growth

The growth of fermented cultures was determined using the direct plate count technique. For this purpose,

PD Agar was used, and the result was expressed as Colony Forming Units per millilitre (CFU/mL).

2.6 Determination of pH and total soluble solid content

pH was measured using a pH meter (Mettler Toledo, Model: Seven Easy GMBH 8603, Switzerland) and the total soluble solid content of all samples was determined with a refractometer (ATAGO, PAL – 3, Japan) whereby the results were reported as °Brix.

2.7 Determination of alcohol and acetic acid content

The alcohol and acetic acid content were determined using Agilent 7890 series gas chromatography equipped with a flame ionization detector (Agilent Technologies, USA). An injection volume of 0.2 µL and a Supelcowax 10 capillary column (30 m × 250 µm × 0.25 µm) was used. The split ratio used was 20:1. The column, injector, detector temperatures were 280°C, 220°C and 250°C, respectively. The carrier gas was hydrogen. The analyses were performed using a temperature programme: 4 mins at 40°C and a linear gradient from 30°C to 240°C for 4 mins.

2.8 Determination of total phenolic content

The total phenolic content (TPC) assay was carried out according to a method by Okmen *et al.* (2009) with some modifications. A total of 5 mL of Folin-Ciocalteu reagent (Merck) and 7.5% Na₂CO₃ (4 mL) were mixed into 1 mL aliquot of each sample. The mixture was allowed to react at room temperature for 2 hrs in the dark. The absorbance was read at 765 nm using a UV-Vis spectrophotometer (VARIAN, Cary 50). The calibration curve was plotted by using 0 to 200 ppm gallic acid as a standard. The standard curve equation was $y = 0.0097x + 0.1152$ with $R^2 = 0.9987$. Results are expressed as mg/mL gallic acid equivalent (GAE).

2.9 Determination of total flavonoid content

The total flavonoid content of samples was determined according to a method described by Chang *et al.* (2002), with some modifications. One mL of each sample was added to 0.3 mL of 5% NaNO₂ solution. After 5 mins incubation, 0.3 mL of 10% AlCl₃ solution was added to the mixture, followed by another incubation for 6 mins. Then, 1 M NaOH solution (2 mL) was added. The final volume of the mixture was brought to 10 mL by using distilled water and the final mixture was allowed to react for 15 mins. The absorbance was measured at 430 nm using a UV-Vis spectrophotometer (VARIAN, Cary 50). Quercetin, at concentrations of 0–100 ppm, was used as a standard. The total flavonoid content was calculated from a quercetin calibration curve ($y = 0.0087x + 0.0328$, with $R^2 = 0.9947$), and the result

was expressed as mg/mL quercetin equivalent (QE).

2.10 Determination of antioxidant activity

2.10.1 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant assay

The assay was carried out with reference to a method by Thaipong *et al.* (2006). Freshly prepared DPPH working solution (2850 µL) was mixed with 150 µL of sample. The concentration of unfermented and fermented extract used in this study was 200 mg/mL. The mixture was allowed to react in the dark for 30 mins. Absorbance was measured at 515 nm using a spectrophotometer (VARIAN, Cary 50). Ascorbic acid was used as the positive control. The percentage of scavenging activity of each sample was determined by using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

2.10.2 Ferric reducing antioxidant power

The FRAP assay was performed as previously described (Benzie and Strain, 1999). Freshly prepared FRAP working solution (2850 µL) was mixed with 150 µL aliquot of each sample (200 mg/mL) and was allowed to react at room temperature in the dark for 30 mins. The absorbance was measured at 593 nm. The standard curve was constructed using 0 to 2000 µM FeSO₄ solution. The ferric reducing antioxidant activity was calculated from a ferrous calibration curve ($y = 1.0986x + 0.1042$, with $R^2 = 0.998$), and the result was expressed as mM/mL ferrous equivalent (FE).

2.11 Phenolic acids and flavonoids analysis

Identification and quantification of phenolic acids and flavonoids in the extracts were conducted using the Acquity™ UPLC system (Waters, Milford, MA, USA), including a binary solvent manager, sample manager and PDA detector. The presence of phenolic acids and flavonoids was determined using a reversed-phase (2.10 × 100 mm, 1.7 µm), Kinetex column and the detector was set at $\lambda = 280$ nm, $\lambda = 330$ nm and $\lambda = 360$ nm. The separation of phenolic acids and flavonoids was made in the gradient condition at 40°C, using a mobile phase made of methanol and acid water (0.3% acetic acid) and the flow rate of the mobile phase was set at 0.4 mL/min.

2.12 Organic acid analysis

Organic acid analysis was performed using a High-Performance Liquid Chromatography (HPLC) Alliance Separation Module (Waters 2695), equipped with a photodiode array detector (Waters 2996). Samples were separated using a column Synergi™ 4 µm Hydro-RP 80 A (LC column 250 × 4.6 mm). The conditions for the

HPLC-analysis were, mobile phase: (A) 1 M KH_2PO_4 and (B) water, flow rate: 0.6 mL/min and column temperature, 30°C. The gradient elution strategy was performed as follows: 100% A was maintained from 0.00 to 30 mins; followed by a linear gradient of A from 100 to 0% for 1 min; then maintained 100% B from 31 mins to 45 mins, linear gradient of A from 0 to 100% also for 1 min; constant ratio of A at 100% for 9 mins. Peak identification was made by comparing retention time and UV spectra at 190, 210 and 254 nm with authentic compounds. The analyses were performed in triplicates.

2.13 Statistical analysis

Mean values and standard deviations were calculated from the data obtained from triplicate experiments. One-way Analysis of Variance (ANOVA) test was used to determine significant differences between variables using Minitab (Version 18) Statistical Software. Differences with a probability value of <0.05 were considered significant. All data were reported as mean \pm standard deviation (SD).

3. Results and discussion

3.1 Alcoholic fermentation of *Ceri Terengganu*

Figure 1 displays the growth of yeast cultures over 10 days of fermentation in the *Ceri Terengganu* medium supplemented with various concentrations of sucrose. The choice of sucrose as the primary fermentable sugar in this study was underpinned by its extensive availability, cost-effectiveness, and established application in diverse fermentative processes. The well-documented efficiency of sucrose metabolism by yeast, as evidenced in prior research, supported its potential to influence fermentative dynamics and product characteristics.

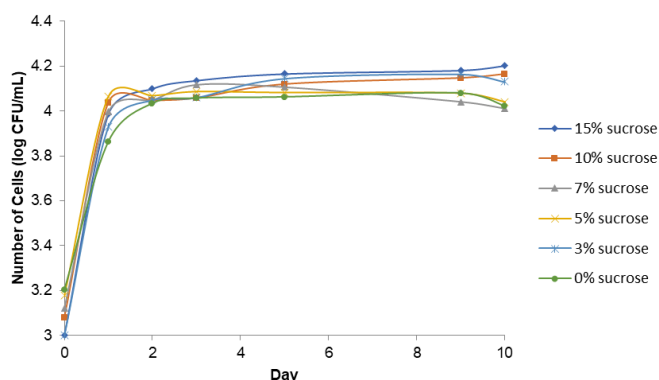


Figure 1. Growth of yeast cultures over 10 days of fermentation in *Ceri Terengganu* with different concentrations of sucrose.

During the 10-day fermentation period, the growth of yeast cultures in the *Ceri Terengganu* medium supplemented with various concentrations of sucrose was

monitored. The total number of yeast cells significantly increased from 3.0 log CFU/mL to 4.2 log CFU/mL, indicating robust yeast proliferation across all samples. Notably, yeast counts peaked between day 3–5 before stabilizing, with no substantial differences among sucrose concentrations by day 10 (Figure 1). This suggests that while sucrose supplementation supports yeast growth, its impact on final cell count is limited.

Concurrently, the pH declined across all samples throughout fermentation, with a slight increase after day 9. The 5% sucrose sample exhibited the most pronounced pH drop, decreasing from 5.95 to 3.59, while higher sucrose concentrations showed a more gradual decline (Figure 2). These results indicated that sucrose concentration influenced pH reduction dynamics, with lower sucrose levels contributing to more rapid acidification.

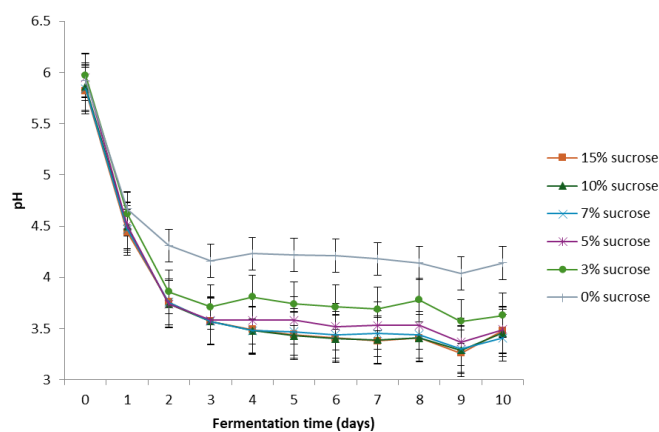


Figure 2. Changes in pH during fermentation of *Ceri Terengganu* with different concentrations of sucrose.

Furthermore, the total soluble solid content, primarily comprising organic sugars such as glucose, sucrose, and fructose, consistently decreased in all samples during fermentation. A notable decline was observed in the sample supplemented with 15% sucrose, with a reduction from 15.4% to 6.1% (Figure 3). This compelling finding supports the active consumption of sucrose by the yeast, leading to a substantial decrease in organic sugars, indicative of yeast's efficient metabolism of sucrose into ethanol and carbon dioxide. This intricate metabolic activity underscores the significant impact of sucrose concentration on the assimilation of organic sugars during fermentation.

The data presented in Figure 4 illustrates the temporal changes in ethanol concentration across the samples during the fermentation of *Ceri Terengganu*. In the absence of sucrose supplementation, ethanol concentration increased until day 2, followed by a subsequent decline. Conversely, the samples supplemented with different concentrations of sucrose demonstrated an overall increase in ethanol

concentration over the fermentation period, with the sample containing 15% sucrose displaying the highest ethanol production. Notably, on day 8 of fermentation, the sample with 15% sucrose supplementation reached an ethanol concentration of 6.41%, indicative of the positive influence of higher sucrose concentrations on ethanol production. These results are consistent with previous studies by Liu *et al.* (2017) and Conterno *et al.* (2013), which also reported heightened ethanol production with increased sucrose concentrations, further supporting the pivotal role of sucrose in promoting ethanol synthesis during fermentation.

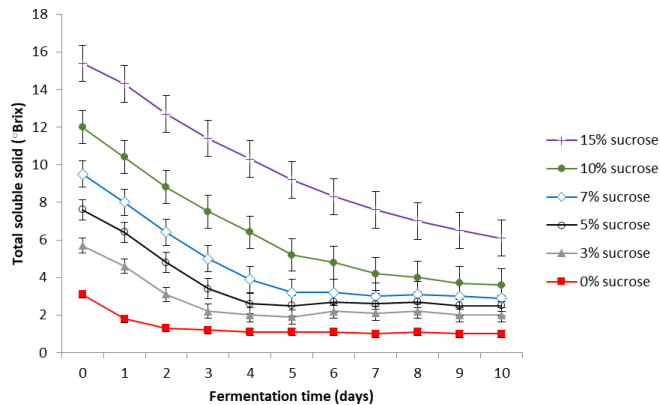


Figure 3. Changes in total soluble solid content during fermentation of Ceri Terengganu with different concentrations of sucrose.

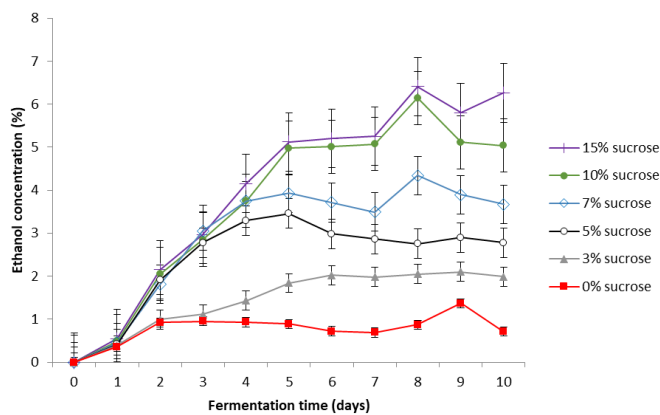


Figure 4. Changes of ethanol concentration during the alcoholic fermentation of Ceri Terengganu with different concentrations of sucrose

Moreover, the observed ethanol concentration of 6.41% in the Ceri Terengganu fermentation aligns with the range of ethanol concentrations attained in mango fermentation. Notably, the ethanol concentration achieved in Ceri Terengganu with 15% sucrose supplementation borders the range obtained from mango fermentation with 20% sucrose (Adebayo-Oyetero *et al.*, 2017), underscoring Ceri Terengganu's capacity to yield comparable ethanol concentrations with lower sucrose levels when compared to mango. Additionally, the timeframe required to achieve this ethanol concentration in Ceri Terengganu was comparatively shorter than that of mango fermentation. These comparisons highlight the

efficacy of sucrose supplementation in enhancing ethanol production during the fermentation of Ceri Terengganu. The findings highlight the relationship between fermentation time, sucrose concentration, and ethanol production, demonstrating the influence of sucrose levels on fermentative dynamics and ethanol synthesis in Ceri Terengganu. Ultimately, these results position Ceri Terengganu as a promising substrate for ethanol production, reinforcing its versatility and potential as a valuable resource in biotechnological applications.

3.2 Acetic fermentation of Ceri Terengganu

The results presented in Figure 5 demonstrate a progressive increase in acetic acid concentration as fermentation time increased. The acetic acid concentration rose from 0.62% to 3.43%, reaching its highest production on day 14 of fermentation. Coinciding with the increase in acetic acid concentration, there was a simultaneous decrease in ethanol concentration from 3.08% to 0.57% after 14 days of fermentation. This can be attributed to the metabolic process of *A. aceti*, which utilizes ethanol as a metabolic substrate for acetic acid production via oxidation. The production of acetic acid from Ceri Terengganu demonstrates its feasibility for use in acetic fermentation processes.

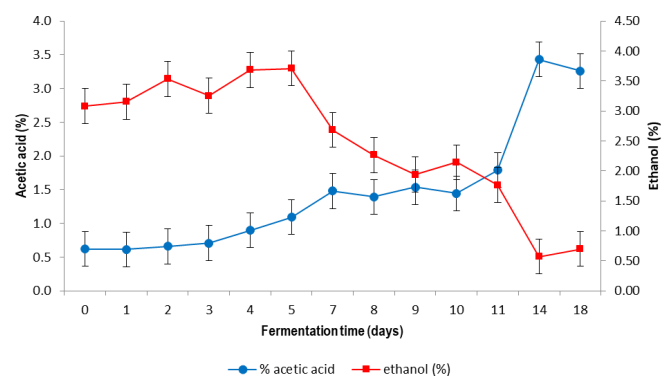


Figure 5. Changes in acetic acid and ethanol concentration during the acetic fermentation of Ceri Terengganu.

However, it should be noted that the final acetic acid concentration observed in this study (3.43%) was lower than that reported in other fruit fermentation products in the literature. For instance, acetic fermentation of mango yielded an acetic acid concentration of 4.5% and jackfruit yielded 4.2% (Buddhika *et al.*, 2021). The differences in acidities among various acetic fermentation products can be attributed to several factors, including variations in raw materials, the quantity of acetic acid bacteria used, fermentation duration, and sugar addition (Li *et al.*, 2014). These variables can influence the metabolic processes and subsequent production of acetic acid during fermentation.

The results illustrated in Figure 6 reveal the variations in pH and total soluble solids content during the acetic fermentation of Ceri Terengganu. After 14 days of fermentation, the pH of the sample decreased from an initial value of 3.87 to a final value of 3.37. This decrease in pH indicates the production of carbon dioxide and an increase in the concentration of acetic acid. The decrease in pH is a characteristic feature of acetic fermentation, as the conversion of ethanol to acetic acid by *A. aceti* results in the release of carbon dioxide and acidification of the solution. Simultaneously, the total soluble solids content of the sample decreased from 9.0°Brix to 8.35°Brix from the first to the tenth day of fermentation. This decline can be attributed to the utilization of sugars by *A. aceti* for growth and the production of acetic acid. The bacteria uptake the sugars present in the substrate and utilize them as a carbon source for their metabolic activities. Alongside acetic acid production, this utilization of sugars contributes to the decrease in total soluble solids content during fermentation.

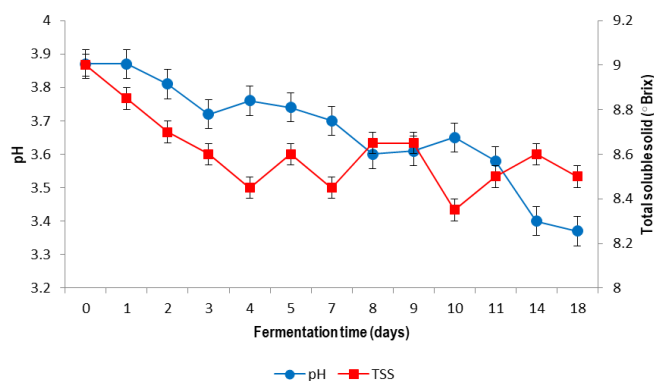


Figure 6. Changes in pH and total soluble solids during acetic fermentation of Ceri Terengganu.

It is important to note that the decrease in total soluble solids content may also be influenced by the production of other functional metabolites during fermentation. These metabolites can contribute to the changes in the composition and characteristics of the fermented product. These findings highlight the dynamic changes in pH and total soluble solids content during the acetic fermentation of Ceri Terengganu. These parameters serve as indicators of the progress and biochemical transformations occurring during fermentation, reflecting the production of acetic acid and other metabolites.

3.3 Total phenolic content, total flavonoid content, and antioxidant activities

Based on Figure 7, the impact of acetic fermentation on the TPC of Ceri Terengganu was evident. The TPC was highest at day 0 (0.79 mg GAE/mL) and remained stable throughout the 14-day fermentation period.

Compared to both unfermented and alcoholic fermentation samples, acetic fermentation resulted in a higher retention of phenolic compounds. The initial increase in TPC may be attributed to the release of bound phenolic compounds from the fruit matrix during maceration, enzyme activation, or potential carryover effects from the preceding alcoholic fermentation.

The enhancement in TPC at the beginning of acetic fermentation, followed by stable retention over time, suggests that this fermentation process not only preserves but also improves the availability of phenolic compounds. This highlights the potential of acetic fermentation in enhancing bioactive compounds, contributing to the functional value of Ceri Terengganu-based products.

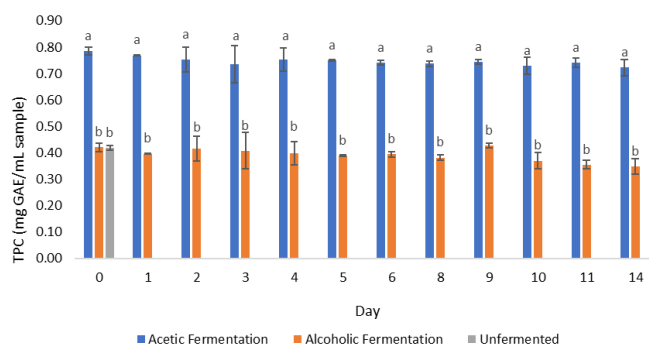


Figure 7. Total phenolics content during fermentation of Ceri Terengganu. Bars with different notations are statistically significantly different ($p < 0.05$).

It is worth noting that the TPC recorded in this acetic fermentation study of Ceri Terengganu surpassed those reported in other acetic fermentation products using different substrates in the existing literature. For example, commercial date yielded a TPC of 0.28 mg GAE/mL, tomato yielded 0.37 mg GAE/mL, unpolished rice yielded 0.73 mg GAE/mL, and persimmon yielded a comparable TPC (Sakanaka and Ishihara, 2008; Lee *et al.*, 2013; Hafzan *et al.*, 2017). These variations in TPC among different fermentation products can be attributed to various factors, including differences in raw materials, fermentation conditions, and specific enzymatic activities, highlighting the need for a more comprehensive understanding of the complex interplay of factors affecting phenolic compound synthesis and stabilization during acetic fermentation of Ceri Terengganu.

In contrast to the TPC, the total flavonoid content in alcoholic fermentation (0.06 mg QE/mL) and acetic fermentation product (0.05 mg QE/mL) was significantly lower ($p < 0.05$) when compared to the unfermented samples (0.19 mg QE/mL) (Figure 8). Interestingly, there was no notable difference in the total flavonoid content between the Ceri Terengganu alcoholic and Ceri Terengganu acetic products. The overall low flavonoid

content across all samples suggests that flavonoids may be naturally limited in Ceri Terengganu or that fermentation could contribute to flavonoid degradation or transformation.

In terms of antioxidant activities, the results of the FRAP assay contradict the results of the DPPH assay. Figure 9 illustrates that there was no significant difference in ferric-reducing antioxidant activity among all samples. This finding can be attributed to the fact that Ceri Terengganu is renowned for its high antioxidant activity. However, when comparing the reducing power of Ceri Terengganu and its fermented products to other fruit acetic fermentation products like buntan pumelo (Jang *et al.*, 2010), orange, mango, cherry, and banana (Coelho *et al.*, 2017), it is evident that Ceri Terengganu and its fermented products demonstrated strong ferric-reducing capacity, suggesting the presence of bioactive compounds capable of donating electrons to neutralize oxidative stress.

On the contrary, Figure 10 demonstrates that unfermented and alcoholic fermentation product samples displayed significantly higher DPPH radical scavenging activity than the acetic fermentation product. This finding aligns with prior research where it was concluded that the antioxidant activity of alcoholic products surpassed that of their unfermented extracts and acetic fermentation products such as blueberry (Su and Silva, 2006) and *Hericium erinaceus* mushroom (Li *et al.*, 2014). While acetic fermentation retained high TPC, its lower DPPH activity suggests that factors beyond TPC, such as specific compound interactions or structural modifications, may influence the antioxidant activity of the final product.

3.4 Phenolic acids and flavonoids composition

In this research study, the focus was on exploring the composition of flavonoids and phenolic acids in Ceri Terengganu juice during alcoholic and acetic fermentation. 7 types of flavonoids (catechin, epigallocatechin, vitexin, rutin, quercetin, luteolin, and apigenin) and 12 types of phenolic acids (gallic, protocatechuic, 4-hydroxybenzoic, 2,5-dihydroxybenzoic, syringic, benzoic, salicylic, ellagic, ferulic, caffeic, chlorogenic, and coumaric acids) were evaluated using UPLC/PDA analysis.

Table 1 shows that during acetic fermentation, there was a successful extraction of epigallocatechin and protocatechuic acid. Interestingly, high concentrations of epigallocatechin (65.57 mg/mL) were detected in the acetic fermentation process, outperforming both alcoholic fermentation and unfermented Ceri Terengganu juice. Epigallocatechin is a type of catechin

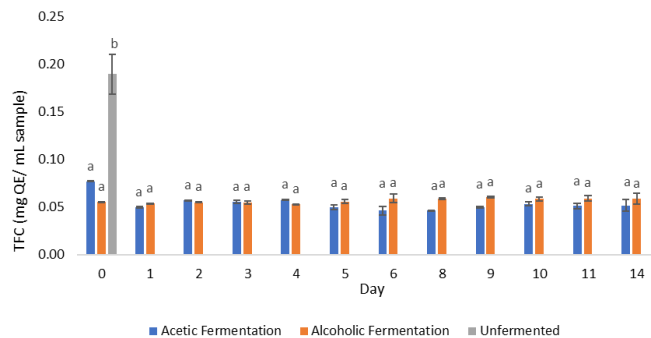


Figure 8. Total flavonoids content of Ceri Terengganu during alcoholic and acetic fermentation. Bars with different notations are statistically significantly different ($p < 0.05$).

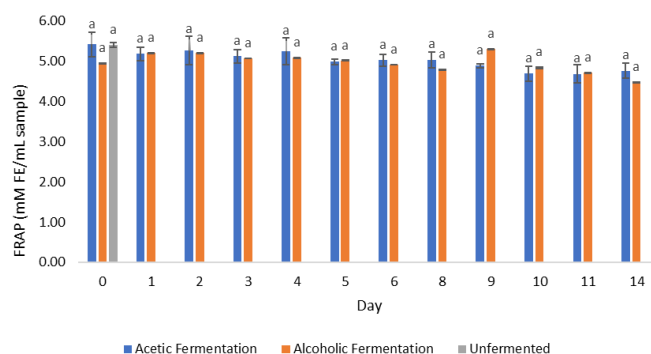


Figure 9. Ferric-reducing antioxidant power (FRAP) during alcoholic and acetic fermentation of Ceri Terengganu. Bars with different notations are statistically significantly different ($p < 0.05$).

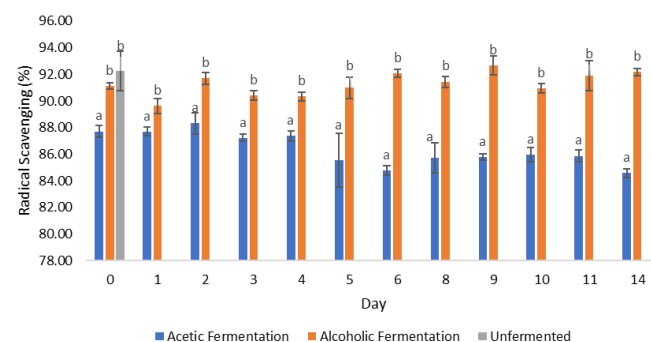


Figure 10. DPPH radical scavenging activity during alcoholic and acetic fermentation of Ceri Terengganu. Bars with different notations are statistically significantly different ($p < 0.05$).

that has been associated with numerous health benefits such as anti-inflammatory, anti-diabetic, anti-obesity, and anti-tumor properties (Min *et al.*, 2014). In fact, the amount of epigallocatechin found in Ceri Terengganu acetic fermentation product in this study was higher than what has been observed in other fruit acetic fermentation products like persimmon (Li *et al.*, 2013), grape, and plum (Jeong *et al.*, 2009).

In addition to the high concentrations of epigallocatechin, the research also revealed that the concentration of protocatechuic acid in acetic fermentation was notably higher than in alcoholic fermentation. The levels ranged from 2.24 mg/mL to

Table 1. Phenolic acids and flavonoids composition of unfermented Ceri Terengganu, Ceri Terengganu alcoholic product and Ceri Terengganu acetic product.

Phenolic acids/flavonoids ($\mu\text{g/mL}$)	Unfermented Ceri Terengganu	Alcoholic fermentation product	Acetic fermentation product
Syringic	0.54 \pm 0.01 ^a	nd	nd
Benzoic	5.15 \pm 0.06 ^a	nd	nd
Ferulic	0.71 \pm 0.02 ^a	nd	nd
Protocatechuic	nd	2.24 \pm 0.04 ^a	12.75 \pm 3.2 ^b
Salicylic	nd	3.4 \pm 0.03 ^a	nd
Epigallocatechin	nd	nd	65.57 \pm 4.1 ^a

Values are presented as mean \pm SD of triplicates. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). nd: not detected.

12.75 mg/mL. Comparing these findings with previous studies, the concentration of protocatechuic acid in Ceri Terengganu acetic fermentation surpassed that of other vinegars, such as apple vinegar (4.1 g/mL) (Nakamura *et al.*, 2010) and shanxi aged vinegar (5.0 g/mL) (Chen *et al.*, 2016). Interestingly, other phenolic acids including syringic, benzoic, and ferulic acids were detected in unfermented Ceri Terengganu juice. However, salicylic acid was only detected during the alcoholic fermentation process. These observations provide valuable insights into the composition of phenolic acids in Ceri Terengganu juice under different fermentation conditions. The higher concentration of epigallocatechin and protocatechuic acid in acetic fermentation compared to other vinegars suggests the potential health benefits of Ceri Terengganu acetic fermentation products. Additionally, the presence of various phenolic acids in unfermented Ceri Terengganu juice highlights the natural abundance of beneficial compounds in the fruit.

3.4 Organic acids composition

The present study also investigated the effects of fermentations on the production of organic acids in Ceri Terengganu juice. In this study, all tested organic acids (lactic, acetic, citric, succinic, L-malic, kojic, and ascorbic acids) were detected in Ceri Terengganu during

acetic fermentation, as shown in Table 2. The oxidation of sugars and alcohols during fermentation primarily accounted for the presence of these organic acids. Acetic acid was found to be the most abundant, followed by citric acid. Interestingly, the concentration of acetic acid increased more than 17-fold in Ceri Terengganu during acetic fermentation in comparison to unfermented Ceri Terengganu (3282.11 $\mu\text{g/mL}$ to 56790.56 $\mu\text{g/mL}$). The amount of acetic acid detected in the acetic fermentation product of this study (56.79 mg/mL) surpassed other studies involving hericium (Li *et al.*, 2014), tomato (Koyama *et al.*, 2017), sugarcane (Chen *et al.*, 2015), and was comparable to other acetic fermentation products such as white wine vinegar, red wine vinegar, alcohol vinegar, malt vinegar, and cider vinegar (Morales *et al.*, 1998; Sáiz-Abajo *et al.*, 2005). The presence of acetic acid in Ceri Terengganu acetic fermentation may contribute to antibacterial effects, as well as potential effects on blood glucose regulation, lipid metabolism, and body weight loss. These findings suggest the promising health-enhancing properties of Ceri Terengganu acetic fermentation products.

In addition, during acetic fermentation, the citric acid content of Ceri Terengganu increased approximately 6-fold (7662.25 $\mu\text{g/mL}$) compared to unfermented Ceri Terengganu (1221.91 $\mu\text{g/mL}$). In alcoholic fermentation,

Table 2. Organic acids composition of unfermented Ceri Terengganu, Ceri Terengganu alcoholic product and Ceri Terengganu acetic product.

Phenolic acids/flavonoids ($\mu\text{g/mL}$)	Unfermented Ceri Terengganu	Alcoholic fermentation product	Acetic fermentation product
Lactic	nd	611.91 \pm 4.92 ^a	318.81 \pm 16.15 ^b
Acetic	3282.11 \pm 66.74 ^a	nd	56790.56 \pm 56.8 ^b
Citric	1221.91 \pm 5.84 ^a	1878.32 \pm 2.47 ^b	7662.25 \pm 64.56 ^c
Succinic	1584.02 \pm 48.06 ^a	747.36 \pm 3.11 ^b	418.91 \pm 11.68 ^c
L – malic	1302.26 19 \pm 6.41 ^a	10031.88 \pm 12.19 ^b	345.87 \pm 28.85 ^c
Kojic	1.09 \pm 0.01 ^a	4 \pm 0.02 ^b	2.44 \pm 0.05 ^a
Ascorbic	nd	9.38 \pm 0.08 ^a	3.08 \pm 0.34 ^b
Oxalic	129.98 \pm 4.16 ^a	nd	nd

Values are presented as mean \pm SD of triplicates. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). nd: not detected.

it increased by approximately 1.5-fold (1878.32 µg/mL). On the other hand, L-malic concentration increased significantly during alcoholic fermentation, by 7.7-fold (from 1302.26 to 10031.88 µg/mL, $p < 0.05$), but decreased to 345.87 µg/mL during acetic fermentation. Both citric and malic acids are commonly found in fermented fruit products, where they act as antimicrobial preservatives. Alcoholic fermentation of Ceri Terengganu juice resulted in the production of lactic acid and ascorbic acid, which were not detected in the unfermented juice. The concentration of lactic acid was found to be 611.91 mg/mL, while ascorbic acid registered a value of 9.38 mg/mL. These organic acids contribute to the flavor profile of the fermented product, imparting a refreshing taste and distinct aroma. Conversely, the concentration of kojic acid, another organic acid of interest, did not display a significant change during the fermentation process. This suggests that either the microorganisms involved in fermentation have a limited ability to produce kojic acid or that its concentration in Ceri Terengganu juice is inherently low. These observations highlight the dynamic changes in organic acid composition during different fermentation processes and suggest the potential preservation and health-promoting properties of Ceri Terengganu fermentation products.

4. Conclusion

In conclusion, Ceri Terengganu has been identified as a viable substrate for two-stage fermentation, involving alcoholic and acetic fermentation. The addition of 15% sucrose in alcoholic fermentation resulted in a maximum ethanol production of 6.41%, indicating the suitability of Ceri Terengganu for this process. Subsequent acetic fermentation with *A. aceti* led to a significant increase in acetic acid concentration from 0.62% to 3.43%. Notably, acetic fermentation enhanced TPC while preserving antioxidant activity in the final product. This suggests the potential for developing beverages with elevated phenolic compounds and enhanced antioxidant properties. Furthermore, the acetic fermentation process yielded higher levels of flavonoids and phenolic acids, notably epigallocatechin and protocatechuic acid. These compounds contribute to the overall health benefits and value of the final product. The study also revealed the dynamic changes in organic acid composition, with acetic and citric acids being the most prominent. These findings highlight the prospect of harnessing Ceri Terengganu for the development of functional products with improved health benefits. Overall, this research establishes the potential of Ceri Terengganu as a substrate for alcoholic and acetic fermentation, paving the way for further exploration and the development of innovative functional products.

Conflict of interest

The authors declare no conflict of interest.

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