

# Exploring the impact of co-fermentation *Saccharomyces cerevisiae* and *Lactobacillus* sp. on stingless bee-honey cider fermentation

Junjira Thipraksa<sup>a</sup>, Panisa Michu<sup>b</sup>, Alisa Kongthong<sup>c</sup>, Pimprapa Chaijak<sup>a,b,\*</sup>

<sup>a</sup>Department of Biology, Faculty of Science, Thaksin University, Phatthalung 93210, Thailand

<sup>b</sup>Department of Biotechnology, Faculty of Science, Thaksin University, Phatthalung 93210, Thailand

<sup>c</sup>Department of Biotechnology, Faculty of Science, Thaksin University, Phatthalung 93210, Thailand

## Article history:

Received: 11 May 2023 / Received in revised form: 30 May 2023 / Accepted: 22 June 2023

## Abstract

Stingless bee honey is a nutritious food that contains a variety of vitamins, minerals, enzymes, and antioxidants. It is known to have higher nutritional and medicinal properties compared to honey produced by other bee species. Cider is a well-known functional drink that contains high antioxidants, which can help protect against cellular damage caused by free radicals. This study aimed to investigate the potential of co-fermentation with yeast (*Saccharomyces cerevisiae*) and bacterium (*Lactobacillus* sp.) in producing high-antioxidant honey cider when compare with standard antioxidant. The results showed that honey cider co-fermented with both microorganisms for 14 days had significantly higher antioxidant activity ( $145.27 \pm 0.20 \mu\text{g TE/mL}$ ) compared to single culture fermentation ( $p < 0.05$ ). Gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of several bioactive compounds in the stingless bee honey cider. These compounds include methylenecyclopropanecarboxylic acid, 2(5H)-furanone, 2-methylbicyclo[4.3.0]non-1(6)-ene, bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl), D-limonene, benzene, 1-(1-butenyl)-4-methoxy, and phytol. These compounds possess various beneficial activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The identification of these compounds in the stingless bee honey cider suggests that it may have potential health benefits beyond its nutritional value. The co-fermentation approach using *S. cerevisiae* and *Lactobacillus* sp. could be considered a promising strategy for developing antioxidant-enriched honey cider with potential health benefits.

**Keywords:** Antioxidant; anticancer; co-culture; dealcoholization; honey cider; stingless bee

## 1. Introduction

Cider, a popular alcoholic beverage, owes its distinct flavor profile to the fermentation process that involves the interaction of yeast and fruit juice. This process generates a diverse range of compounds, such as ethanol, higher alcohols, ethyl acetate (EA), and ethyl formate, collectively contributing to the characteristic aroma of cider [1]. Recent research has highlighted the potential benefits of co-culturing non-*Saccharomyces* yeasts alongside the primary fermenting agent, *Saccharomyces cerevisiae*, to enhance the complexity of alcohol aromas. While *Saccharomyces cerevisiae* plays a crucial role in driving the alcoholic fermentation process, it is now understood that the co-presence of other yeast strains can significantly impact the sensory characteristics of the final product, particularly its aroma [2]. During the fermentation of cider, sugars are converted into ethanol and carbon dioxide, accompanied by the production of various metabolites that greatly influence the sensory attributes of the beverage, including its aroma. The development of aromas in alcoholic beverages is a result of intricate interactions within multiple biosynthetic pathways [2,3]. By exploring the co-culturing approach, researchers aim to broaden the range of aromatic

compounds in cider, thereby offering consumers a more diverse and enjoyable sensory experience. This article will delve into the potential advantages and implications of co-culturing non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae* in the production of cider, shedding light on the underlying mechanisms and highlighting the exciting possibilities for aroma enhancement in this popular alcoholic beverage.

Moreover, the selection of yeast strain profoundly impacts the aromatic complexity of the resulting cider [4–7]. Spontaneous fermentation, which involves the involvement of non-*Saccharomyces* yeasts during the initial stages, was a commonly practiced method in the past [3]. In a recent study by Anne Gschaedler and colleagues (2021), the utilization of diverse non-*Saccharomyces* species for fermenting apple juice was proposed, emphasizing the importance of specific nutrients for successful alcoholic fermentation and the production of desirable volatile compounds in cider production [8]. Consequently, the careful selection of yeast strains has become increasingly critical in the cider industry.

Yeasts, ubiquitous microorganisms found in various natural environments, establish symbiotic relationships with plants and insects. Within the context of cider production, yeasts found in flowers and fruits play a pivotal role in generating volatile compounds, including ethanol, ethyl acetate (EA), isoamyl acetate, and 2-phenylethyl acetate (PEA). These compounds serve as attractants for insects, playing a crucial role in the

\* Corresponding author.

Email: [chaijak.pimprapa@gmail.com](mailto:chaijak.pimprapa@gmail.com)

<https://doi.org/10.21924/cst.8.1.2023.1185>

propagation of plants [9,10]. Of particular interest are the aroma-contributing compounds, such as PEA, which imparts honey- and rose-like aromas, and its precursor, 2-phenylethanol (PE), known for its rose-like scent. Both PE and PEA have been classified as safe additives (Generally Recognized As Safe - GRAS) and find widespread application in the food and cosmeceutical industries [11,12].

Alcoholic beverages have long been associated with various health concerns, primarily due to the presence of ethanol, a primary carcinogen produced during the fermentation process carried out by *Saccharomyces cerevisiae*. Even small amounts of ethanol consumption can increase the risk of cancer, and a universally recognized safety standard for ethanol intake is yet to be established [13]. In response to these health concerns, there has been a significant surge in demand for low- and non-alcoholic beverages as consumers increasingly prioritize healthier diets. Consequently, scientific research has focused on developing non-alcoholic fermented juices as a healthier alternative to traditional alcoholic beverages.

Numerous studies have explored different methods for removing ethanol from fermented juices, including distillation, membrane filtration, membrane reverse osmosis, and nanofiltration [14]. While these methods preserve the functional substances and nutrients in the beverages, they also result in the loss of aroma components, thereby impacting the overall taste and quality. Despite these challenges, scientific researchers continue to explore ways to enhance the sensory appeal of non-alcoholic fermented juices, providing a healthier and more enticing option for consumers seeking to avoid the negative health consequences associated with ethanol consumption [15].

In recent years, probiotics, live bacteria that offer health benefits when consumed, have garnered significant scientific interest. Among the various probiotics, *Lactobacillus* sp. has proven to be particularly efficient in fermenting healthy beverages [16]. Ingesting *Lactobacillus* sp. has demonstrated a range of positive effects on the human body. For instance, a study conducted by Swarna et al. highlighted that the consumption of *Lactobacillus* sp. promotes the dynamic balance of active microorganisms within the host body, leading to improvements in human immunity and the enhancement of intestinal flora [17]. These findings hold significant implications for the development of functional foods and probiotic supplements that can offer targeted health benefits to consumers. Furthermore, they underscore the importance of ongoing scientific research into probiotics and their effects on the human body as we strive to better understand their mechanisms of action and develop new approaches to improving human health [18].

One specific application of co-fermentation involving *Saccharomyces cerevisiae* and *Lactobacillus* sp. is in cider production, where it can result in a complex flavor profile and improved sensory attributes. *Saccharomyces cerevisiae* contributes to the production of ethanol, providing the characteristic alcoholic taste, while *Lactobacillus* sp. produces lactic acid, adding acidity and influencing the overall flavor of the cider. Additionally, the metabolic activities of both microorganisms can generate other compounds, such as esters, higher alcohols, and volatile aromas, further enhancing the sensory characteristics of the final product [19].

The aim of this research was to assess the effects of co-fermenting *Saccharomyces cerevisiae* and probiotic *Lactobacillus* sp. on the overall quality of stingless honey cider. The specific focus was on developing a cider that exhibits high antioxidant content, thereby offering potential health benefits.

## 2. Materials and Methods

### 2.1. Chemicals and materials

The *Lactobacillus* medium de Man, Rogosa and Sharpe medium (MRS) was purchased from Sigma-Aldrich (Sigma-Aldrich, MO, USA). The potato dextrose agar (PDA) and potato dextrose broth (PDB) were purchased from Himedia (Himedia, Maharashtra, India). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and buffer were purchased from Sigma-Aldrich (Sigma-Aldrich, MO, USA).

The stingless bee honey derived from citrus plants was collected using a sterile technique and procured from a local beekeeper situated in the province of Phatthalung, Thailand. The coconut water was acquired from Malee Capital Company Ltd. in Bangkok, Thailand. Both the honey and coconut water were subjected to storage at a temperature of -25 °C until their intended use. The properties of coconut water was shown in Table 1 based on Manufacturer's data.

### 2.2. Starter strain and culture maintenance condition

The yeast *Saccharomyces cerevisiae* isolated from honey, and the probiotic bacterium *Lactobacillus* sp. were obtained from the Department of Biology, Faculty of Science, Thaksin University, Thailand. The yeast was maintained on PDA plates, while the bacterium was maintained on MRS agar plates. All cultures were stored at a temperature of 4 °C until their intended use. The morphology of *Saccharomyces cerevisiae* and *Lactobacillus* sp. shows in Figure 1.

Table 1. Properties of coconut water used in this experiment based on manufacturer's data

Composition	Amount	Unit
Energy	220	kcal/L
Carbohydrates	55	g/L
Sugars	49	g/L
Sodium (Na)	210	mg/L
Potassium (K)	1,990	mg/L
Phosphorus (P)	90	mg/L
Calcium (Ca)	170	mg/L

### 2.3. Must preparation

To produce a cider beverage, a solution was prepared by diluting honey in coconut water to achieve a concentration of 37% w/v. The mixture was homogenized according to the method described in Mendes-Ferreira et al. [20]. Insoluble materials were separated from the mixture through centrifugation at 12,000 rpm for 10 min, resulting in a clarified honey-must. To adjust the titratable acidity, 5 g/L of potassium tartrate (Sigma-Aldrich, MO, United States) was added. The pH was subsequently adjusted to 3.7 using malic acid (Merck,

Darmstadt, Germany). The nitrogen content was adjusted to 267 mg/L using di-ammonium phosphate (DAP, Himedia, Maharashtra, India). The parameters of the solution, including °Brix, pH and total acidity were measured before and after the adjustments. The honey must was then pasteurized by heating them to 65 °C for 10 min before being rapidly cooled. No sulfur dioxide was added to the honey-musts

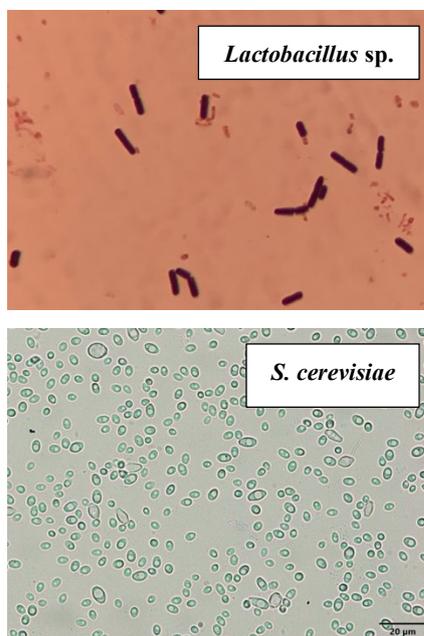


Fig. 1. The morphology of *Saccharomyces cerevisiae* and *Lactobacillus* sp.

#### 2.4. Fermentation condition

A co-culture consisting of yeasts and bacteria was prepared by combining 20 mL of a mixture containing a cell concentration ratio of 1:1 ( $1 \times 10^8$  cell/mL:  $1 \times 10^8$  cell/mL). The co-culture was then introduced into a pretreated honey must. To establish a reference point, a single culture of the respective yeast and bacteria were also introduced into the honey must at a concentration of  $1 \times 10^8$  cell/mL. The Brix of all solutions was monitored.

After a fermentation period of 14 days, the honey cider was separated from the cultures using centrifugation at 12,000 rpm, 4 °C for 15 min. The resulting honey cider was collected and stored in brown airtight glass containers at 4 °C to prevent interference from oxygen and light. The stored honey cider was then subjected to physicochemical analysis.

#### 2.5. Antioxidant activity

The evaluation of the antioxidant capacity of honey cider was conducted by assessing its DPPH radical scavenging activity, employing a modified method based on Kim et al. [21]. A volume of 0.1 mL of the cider sample was combined with 3.9 mL of a methanolic solution containing DPPH at a concentration of 25 mg/mL. The resulting mixture was allowed to react for a period of 15 min. Control tests were performed concurrently using deionized water in lieu of the cider sample. The absorbance of each mixture was measured using a UV/Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at a wavelength of 515 nm, with methanol serving as the reference.

Each measurement was carried out in triplicate to ensure the accuracy and precision of the results. The antioxidant capacity was quantified and expressed in terms of micrograms of trolox equivalents (TE) per milliliter (mL), providing a standardized measure of antioxidant activity in the honey cider.

#### 2.6. GC-MS analysis

The volatile composition of honey cider was determined using gas chromatography-mass spectrometry (GC-MS) analysis (Agilent 7890B/5977, Agilent, CA, United States) following the method described by Lorenzini et al. [22]. Briefly, 10 µL of an internal standard solution (2-octanol at 420 mg/L in ethanol) was added to 50 mL of honey cider diluted with 50 mL of distilled water to prepare the samples. The samples were then loaded onto a solid-phase extraction (SPE) cartridge that had been activated with 20 mL of methanol and equilibrated with 20 mL of MilliQ water. The cartridge was washed with 15 mL of water, and the elution of the volatile compounds was completed with 10 mL of dichloromethane. The collected organic layer was dried with sodium sulfate, concentrated to approximately 200 µL under a gentle nitrogen stream, and subjected to GC-MS analysis using an HP 7890 A gas chromatograph coupled to a 5977 B quadrupole mass spectrometer. Separations were carried out on a DB-WAX UI capillary column (30 m × 0.25 mm, 0.25 µm film thickness) (Agilent, CA, United States) using helium as the carrier gas at a flow rate of 1.2 mL/min.

Gas Chromatography (GC) analysis was conducted using a GC oven, with temperature settings as follows: an initial temperature of 40 °C maintained for 3 min, followed by a programmed increase to 230 °C at a rate of 4 °C per minute. The final temperature of 230 °C was maintained for a duration of 20 min. Mass spectrometry analysis was performed in electron ionization (EI) mode with an energy of 70 eV. The ion source temperature was set at 250 °C, while the quadrupole temperature was maintained at 150 °C.

For quantification purposes, mass spectra were acquired using Selected Ion Monitoring (SIM) mode, allowing for precise measurement of specific ions of interest. The identification and quantification of volatile compounds were accomplished by employing commercial standards as reference samples. These standards served as a basis for comparison and calibration, enabling the determination of the composition and concentration of the volatile compounds present in the samples under investigation.

#### 2.7. Statistic analysis

The statistical analysis, specifically ANOVA analysis was performed using IBM SPSS Statistics software, version 7.0.1.0.237 (IBM, NY, United States).

### 3. Results and Discussion

#### 3.1. Oenological analysis

Figure 2 presents the visual representation of the stingless bee honey cider produced in this study, both before and after the fermentation process. It is evident from the figure that the

color of the honey must has undergone a noticeable change following fermentation. This finding is significant and will be further discussed in the results section of the article. Figure 3 showed Brix values of ciders fermented using co-culture (*Saccharomyces cerevisiae* and *Lactobacillus* sp.), *Saccharomyces cerevisiae* and *Lactobacillus* sp.

Table 2 provides a summary of the physicochemical values of the ciders. Significant variations in sugar residues were observed among the different strains used. However, no significant differences were observed between the single yeast strain and co-culture groups for the same yeast strain.

Regarding the Brix value, minimal changes were observed from day 0 to day 2 in the honey must fermentation by *Saccharomyces cerevisiae* and the co-culture. However, a significant change was observed on day 4. In contrast, the honey must fermented by *Lactobacillus* sp. showed no significant change in the Brix value. These findings will be further elaborated upon in the results section of the article.

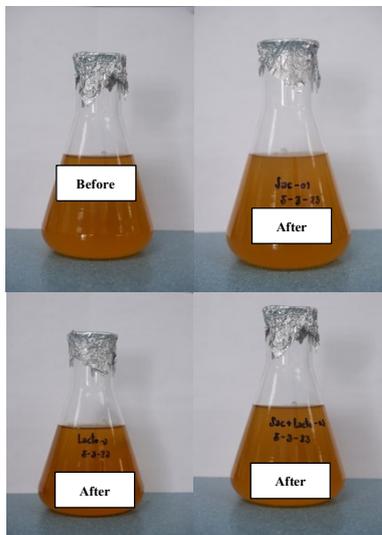


Fig. 2. Stingless bee honey cider fermented by *Saccharomyces cerevisiae* (Sac), *Lactobacillus* sp. (Lac) and co-culture, *Saccharomyces cerevisiae* and *Lactobacillus* sp.

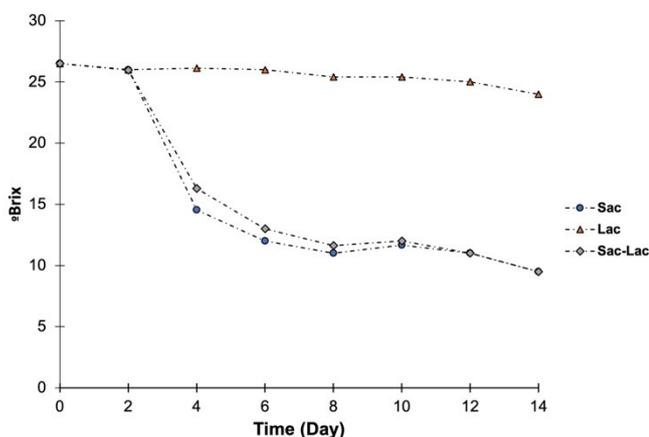


Fig. 3. The brix value of stingless bee honey cider for 14 days of fermentation (Sac: *Saccharomyces cerevisiae*; Lac: *Lactobacillus* sp.; Sac-Lac: *Saccharomyces cerevisiae* and *Lactobacillus* sp.) (n=3)

The utilization of sugar is an important indicator of the ability of cultures to carry out alcoholic fermentation and transform substrates [23]. Stingless bees use a different strategy

for honey storage. They dehydrate the honey to a specific level, as reported by Vit et al. [24] and Souza et al. [25]. During storage, microorganisms, particularly *Bacillus* bacteria and yeasts, consume a portion of the sugar, leading to the production of alcohol via anaerobic fermentation. Subsequently, aerobic fermentation transforms this alcohol into acetic acid. Furthermore, other types of non-alcoholic fermentation can transform sugar into various acids and other by-products [26]. Balogu et al. used *Saccharomyces cerevisiae* to produce honey-coconut wine. These wines have stable acidity, but their sweetness (ranging from dry to semi-sweet), calorie content (ranging from very low to moderate), and alcohol content (ranging from low to high) depend on the composition of honey in the blend. Developing models that establish correlations (>95%) between microbial kinetics, wine composition (honey : coconut water), and wine quality would provide a valuable analytical tool for predicting the honey-coconut wine-making process with reliable precision [27].

Furthermore, a previous study has shown that the probiotic bacterium *Lactobacillus* sp. was successfully employed for the dealcoholization of fermented beverages through co-culturing with the alcoholic yeast *Saccharomyces cerevisiae* [28], which is consistent with our findings.

Table 2. Physicochemical characteristics of stingless honey cider (n=3)

Parameter	Control	Sac	Lac	Sac-Lac
Reducing sugar (g/L)	294.3±0.2	98.1±0.5	263.8±0.3	98.1±0.5
pH	4.4±0.1	3.65±0.2	3.5±0.1	3.48±0.3
Tatrabl acidity (g/L tartaric acid)	0.5±0.1	3.0±0.1	7.1±0.0	7.4±0.3
Alcohol (%)	0.0±0.0	11.3±0.2	0.0±0.0	0.5±0.2

(Sac: *Saccharomyces cerevisiae*; Lac: *Lactobacillus* sp.; Sac-Lac: *Saccharomyces cerevisiae* and *Lactobacillus* sp.)

### 3.2. Antioxidant activity

The antioxidant activity of honey ciders produced by co-fermentation with *Saccharomyces cerevisiae* and *Lactobacillus* sp. was assessed by means of DPPH radical scavenging assay. The results showed that after 14 days of co-fermentation, the ciders exhibited the highest antioxidant activity, which was measured at 0.00±0.00, 5.68±0.11, 39.13±0.12, 43.89±1.21, 56.35±0.10, 83.20±0.56, 122.01±0.35 and 145.27 ± 0.20 µg TE/mL on day-0, day-2, day-4, day-6, day-8, day-10, day-12 and day-14, respectively. This value was significantly higher than that observed in ciders fermented on day-14 with either single yeast or bacterium strains, as shown in Figure 4.

On the other hand, it has been observed that stingless bee honey which exhibits remarkable antioxidant properties, can also display an appealing characteristic of increased levels of 2-phenylethanol, resulting in a rose-like flavor and aroma when subjected to fermentation with the yeast strain *Torulasporea delbrueckii* [29]. In Fiorda et al., the antioxidant activity of honey-based kefir beverage was found to be higher when it was fermented using probiotic strains of *Lactobacillus statsumensis*, *Leuconostoc mesenteroides*, *Bacillus megaterium*, *Saccharomyces cerevisiae* and *Lachancea*

*fermentati*. These findings suggest that honey can be an ideal alternative substrate for the production of functional cultured beverages, especially for individuals who follow a vegan diet or are lactose intolerant [30].

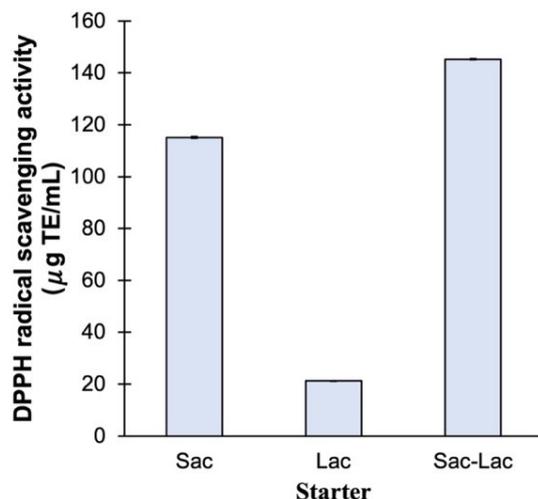


Fig. 4. Changes in DPPH radical scavenging activity in different stingless bee honey cider following a 14 days of single and co-fermentation. (Sac: *Saccharomyces cerevisiae*; Lac: *Lactobacillus* sp.; Sac-Lac: *Saccharomyces cerevisiae* and *Lactobacillus* sp.) (n=3)

### 3.3. Cider composition

In this study, the impact of fermentation conditions on the aromatic composition of honey cider was evaluated. Specifically, the co-culture fermented cider was subjected to GC-MS analysis at the end of fermentation to investigate any potential changes in its aromatic profile. The chromatogram presented in Figure 5 depicts the results of the fermentation process of stingless bee-honey cider using a co-culture of *Saccharomyces cerevisiae* and *Lactobacillus* sp. The chromatogram shows the separation and detection of the various chemical components present in the fermented beverage.

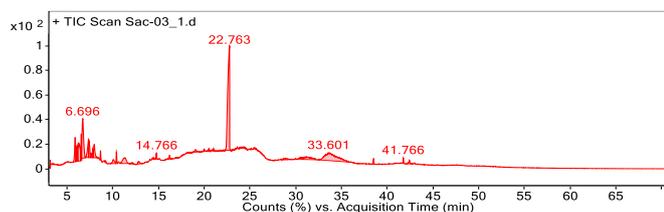


Fig. 5. Chromatogram of compounds elucidated from stingless bee-honey cider using GC-MS analysis

The chromatogram peak analysis of the stingless bee-honey cider solution revealed the presence of 7 compounds. These compounds were identified based on their retention times and mass spectra, and their structures were confirmed by comparison with authentic standards. The first compound detected was methylenecyclopropanecarboxylic acid (Figure 6 (a)), which has a retention time of 6.546 and 6.698 min. The second compound detected was 2(5H)-furanone (Figure 6(b)), which has a retention time of 7.333 min. The third compound detected was 2-methylbicyclo[4.3.0]non-1(6)-ene (Figure 6 (c)), which has a retention time of 7.793 min.

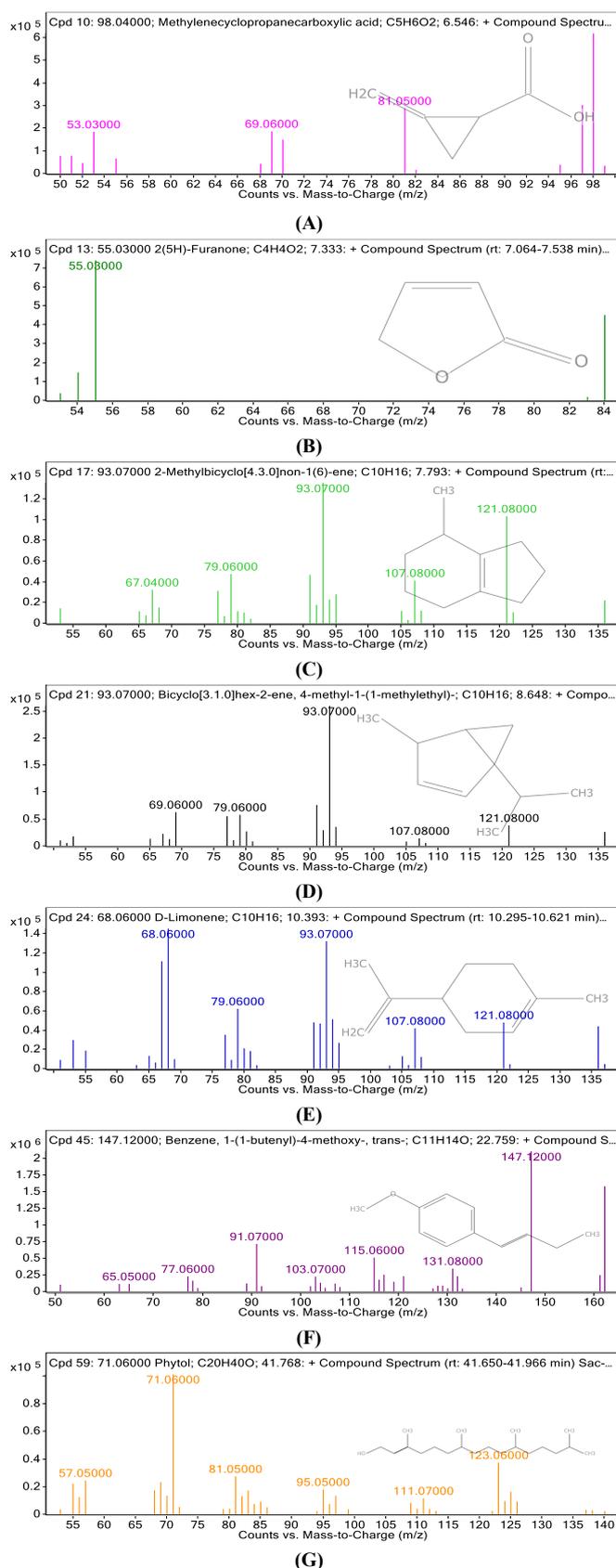


Fig. 6. Methylenecyclopropanecarboxylic acid (a), 2(5H)-furanone (b), 2-methylbicyclo[4.3.0]non-1(6)-ene (c), Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl) (d), D-limonene (e), Benzene, 1-(1-butenyl)-4-methoxy (f), and Phytol (g) found in stingless bee-honey cider

The fourth compound detected was bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl) (Figure 6(d)), which has a retention time of 8.648 min. The fifth compound detected

was D-limonene (Figure 6(e)), which has a retention time of 10.393 min. The sixth compound detected was benzene, 1-(1-butenyl)-4-methoxy (Figure 6(f)), which has a retention time of 22.759 min. The seventh and final compound detected was phytol (Figure 6(g)), which has a retention time of 41.768 min. The identification of these compounds and their respective retention times can be used to determine the chemical composition and quality of the stingless bee-honey cider solution.

Table 3 presents a summary of the chemical compounds identified in the analysis of the stingless bee-honey cider solution and their respective biological activities.

Table 3. Chemical compounds identified in the analysis of the stingless bee-honey cider and their respective biological activities

Compound	R time	Molecular formula	Biological activity	Ref.
Methylenecyclopropane carboxylic acid	6.546, 6.698	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	Antimicrobial activity	[31]
2(5H)-Furanone	7.333	C <sub>4</sub> H <sub>4</sub> O <sub>2</sub>	Anticancer activity	[32]
2-Methylbicyclo[4.3.0]non-1(6)-ene	7.793	C <sub>10</sub> H <sub>16</sub>	Antimicrobial activity	[33]
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)	8.648	C <sub>10</sub> H <sub>16</sub>	Anticancer activity, Anti-inflammatory activity	[34]
D-Limonene	10.393	C <sub>10</sub> H <sub>16</sub>	Antimicrobial activity	[35]
Benzene, 1-(1-butenyl)-4-methoxy	22.759	C <sub>11</sub> H <sub>14</sub> O	Antioxidant activity	[36]
Phytol	41.768	C <sub>20</sub> H <sub>40</sub> O	Anticancer activity	[32]

#### 4. Conclusion

In conclusion, this study demonstrated that co-fermentation of yeast (*Saccharomyces cerevisiae*) and bacterium (*Lactobacillus* sp.) in the production of stingless bee honey cider can significantly enhance its antioxidant activity. The honey cider co-fermented with both microorganisms exhibited a notably higher level of antioxidants compared to single culture fermentation. The gas chromatography-mass spectrometry (GC-MS) analysis further revealed the presence of various bioactive compounds with beneficial properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. These findings suggest that stingless bee honey cider may offer potential health benefits beyond its nutritional value.

The identification of these bioactive compounds highlights the potential of stingless bee honey cider as a functional beverage. The co-fermentation approach using *Saccharomyces cerevisiae* and *Lactobacillus* sp. presents a promising strategy for developing antioxidant-enriched honey cider with enhanced health-promoting properties. Further research and development in this area can lead to the production of novel and nutritious beverages that contribute to overall well-being. Overall, this study contributes to the growing body of knowledge regarding the utilization of stingless bee honey and the potential benefits

of co-fermentation in cider production. It provides insights into the bioactive compounds present in stingless bee honey cider and their potential roles in promoting health. These findings open up avenues for further exploration and application of this unique beverage in the development of functional foods and beverages with enhanced antioxidant properties and potential health benefits.

#### Acknowledgements

This work was supported by Thailand Science Research and Innovation Office of National Higher Education Science Research and Innovation Policy Council, Thaksin University (research project grant) Fiscal Year 2023.

#### References

1. F. Cousin, M. Schlüsselhuber, M. Dalmaso, J. M. Laplace, M. Cretenet. *Microorganisms in fermented apple beverages: Current knowledge and future directions*, *Microorganisms*. 5 (2017) 39.
2. J. H. Swiegers, I. S. Pretorius. *Yeast modulation of wine flavor*, *Adv. Appl. Microbiol.* 57 (2005) 131-175.
3. W. E. Kiyat, S. D. Astuti, S. Budijanto, E. Syamsir. *Potential of lacto-N-biose I as a prebiotic for infant health: A review*, *Commun. Sci. Technol.* 6 (2021) 18-24.
4. M. Gonzalez Flores, M. E. Rodriguez, A. C. Origone, J. M. Oteiza, A. Querol, C. A. Loprs. *Saccharomyces uvarum isolated from patagonian ciders shows excellent fermentative performance for low temperature cidermaking*, *Food Res. Int.* 126 (2019) 108656.
5. H. Guichard, P. Poupard, L. Legoahec, M. Millet, R. Bauduin, J. M. Le Quere. *Brettanomyces anomalus, a double drawback for cider aroma*, *LWT.* 102 (2019) 214-222.
6. J. Lleixa, V. Martin, M.D.C. Portillo, F. Carrau, G. Beltran, A. Mas. *Comparison of fermentation and wines produced by inoculation of Hanseniaspora vineae and Saccharomyces cerevisiae*, *Front. Microbiol.* 7 (2016) 338.
7. W. K. Shi, J. Wang, F. S. Chen, X. Y. Zhang. *Effect of Issatchenkia terricola and Pichia kudriavzevii on wine flavor and quality through simultaneous and sequential co-fermentation with Saccharomyces cerevisiae*, *LWT.* 116 (2019) 108477.
8. A. Gschaedler, L. E. Iniguez-Munoz, N. Y. Flores-Flores, M. Kirchmayr, M. Arellano. *Use of non-Saccharomyces yeasts in cider fermentation: Importance of the nutrients addition to obtain an efficient fermentation*, *Int. J. Food Microbiol.* 347 (2021) 109169.
9. M. C. Dzialo, R. Park, J. Steensels, B. Lievens, K. J. Verstrepen. *Physiology, ecology and industrial applications of aroma formation in yeast*, *FEMS Microbiol. Rev.* 41 (2017) S95-S128.
10. A. A. Madden, M. J. Epps, T. Fukami, R. E. Irwin, J. Sheppard, D. M. Sorger, R. R. Dunn. *The ecology of insect-yeast relationships and its relevance to human industry*, *Proc. R. Soc. B. Biol. Sci.* 102 (2018) 20172733.
11. O. Martinez-Avila, A. Sanchez, X. Font, R. Barrena. *Bioprocesses for 2-phenylethanol and 2-phenylethyl acetate production: Current state and perspectives*, *Appl. Microbiol. Biotechnol.* 102 (2018) 9991-10004.
12. X. Qian, W. Yan, W. Zhang, W. Dong, J. Ma, K. Ochsenreither, M. Jiang, F. Xin. *Current status and perspectives of 2-phenylethanol production through biological processes*, *Crit. Rev. Biotechnol.* 39 (2019) 235-248.
13. A. M. Wood, S. Kaptoge, A. S. Butterworth. *Risk thresholds for alcohol*

- consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies, *Lancet*. 391 (2018) 1513-1523.
14. D. Mangindaan, K. Khoiruddin, I. G. Wenten. *Beverage dealcoholization processes: Past, present, and future*. *Trend Food Sci. Technol.* 71 (2018) 36-45.
  15. Y. Li, T. T. H. Nguyen, J. Jin, J. Lim, J. Lee, M. Piao, I. K. Mok, D. Kim. *Brewing of glucuronic acid-enriched apple with enhanced antioxidant activities through the co-fermentation of yeast (*Saccharomyces cerevisiae* and *Pichia kudriavzevii*) and bacteria (*Lactobacillus plantarum*)*, *Food Sci. Biotechnol.* 30 (2021) 555-564.
  16. S. Grover, H. M. Rashmi, A. K. Srivastava, V. K. Batish. *Probiotics for human health – new innovations and emerging trends*, *Gut Pathog.* 4 (2012) 15.
  17. S. R. Swarna, N. N. Srimathi, R. Madhavan, S. Gomathi. *Performance of extended spectrum beta lactamase (ESBL) screening agar in various clinical specimens*, *Indian J. Med. Res.* 141 (2015) 481.
  18. H. Li, J. Huang, Y. Wang, X. Wang, Y. Ren, T. Yue, Z. Wang, Z. Gao. *Study on the nutritional characteristics and antioxidant activity of dealcoholized sequentially fermented apple juice with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* fermentation*, *Food Chem.* 363 (2021) 130351.
  19. X. Chen, M. Lin, L. Hu, T. Xu, D. Xioang, L. Li, Z. Zhao. *Research on the effect of simultaneous and sequential fermentation with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* on antioxidant activity and flavor of apple cider*, *Fermentation.* 9 (2023) 102.
  20. A. Mendes-Ferreira, F. Cosme, C. Barbosa, V. Falco, A. Ines, A. Mendez-Faia. *Optimization of honey-must preparation and alcoholic fermentation by *Saccharomyces cerevisiae* for mead production*, *Int. J. Food Microbiol.* 144 (2010) 198-198.
  21. H. Kim, J. S. Kim, Y. Jeong, J. E. Kim, N. S. Paek, C. H. Kang. *Antioxidant properties of *Lactobacilli* and *Biofidobacteria* of human origins*, *Biotechnol. Bioproc. Eng.* 25 (2020) 421-430.
  22. M. Lorenzini, B. Simonato, D. Slaghenaufi, M. Ugliano, G. Zapparoli. *Assessment of yeasts for apple juice fermentation and production of cider volatile compounds*, *LWT.* 99 (2019) 224-230.
  23. Z. Wang, K. Xu, R. Cai, T. Yue, Y. Yuan, Z. Gao. *Construction of recombinant fusant yeasts for the production of cider with low alcohol and enhanced aroma*, *European Food Res. Technol.* 246 (2020) 745-757.
  24. P. Vit, S. Bogdanov, V. Kilchenman. *Composition of venezuelan honeys from stingless bees (*Apidae: Meliponinae*) and *Apis mellifera* L.*, *Apidologie.* 25 (1994) 278-288.
  25. B. Souza, D. Poubik, O. Barth, T. Heard, E. Enrlquez, C. Carvalho, J. Villas-Boas, L. Marchini, J. Locatelli, L. Persano-Odda, L. Almeida-Muradian, S. Bogdanov, P. Vit. *Composition of stingless bee honey: setting quality standards*, *Interciencia.* 31 (2006) 867-875.
  26. M. Gilliam, D. W. Roubik, B. J. Lorenz. *Microorganisms associated with pollen, honey, and brood provisions in the nest of a stingless bee, *Meliposa fasciata**, *Apidologie.* 21 (1990) 89-90.
  27. T. V. Balogu, O. Towobola. *Production and quality of wine from honey and coconut milk using *Saccharomyces cerevisiae**, *Fermentation.* 3 (2017) 16.
  28. H. Li, J. Liang, M. Han, X. Wang, Y. Ren, Y. Wang, J. Huang, S. Li, C. Liu, Z. Wang, T. Yue, Z. Gao. *Sequentially fermented dealcoholized apple juice intervenes fatty liver induced by high-fat diets via modulation of intestinal flora and gene pathways*, *Food Res. Int.* 156 (2022) 111180.
  29. C. Sooklim, W. Samakkarn, A. Thongmee, O. Duangphakdee, N. Soontorngun. *Enhanced aroma and flavor profile of fermented *Tetragonula pagdeni* Schawarz honey by a novel yeast *T. delbrueckii* GT-ROSE1 with superior fermentability*, *Food Biosci.* 50 (2022) 102001.
  30. F. A. Fiorda, G. V. D. M. Pereira, V. Thomaz-Soccol, A. P. Medeiros, S. K. Rakshit, C. R. Soccol. *Development of kefir-based probiotic beverages with DNA protection and antioxidant activities using soybean hydrolyzed extract, colostrum and honey*, *LWT.* 68 (2016) 690-697.
  31. D. T. W. Chu, A. K. Claiborne, J. J. Clement, J. J. Plattner. *Syntheses and antibacterial activity of novel 6-fluoro-7-(gem-disubstituted piperazin-1-yl)-quinolines*, *Can. J. Chem.* 70 (1992) 1328.
  32. P. K. Anoor, A. N. Yadav, K. Rajkumar, R. Kande, C. Tripura, K. S. Naik, S. Burgula. *Methanol extraction revealed anticancer compounds quinic acid, 2(5H)-furanose and phytol in *Andrographis paniculata**, *Mol. Clin. Oncol.* 17 (2022) 151.
  33. P. Salvi, G. Kumar, N. Gandass, K. Kajal, A. Verma, S. Rajarammohan, N. Rai, V. Gautam. *Antimicrobial potential of essential oils from aromatic plant *Ocimum* sp.: A comparative biochemical profiling and in-silico analysis*, *Agronomy.* 12 (2022) 627.
  34. G. Chen, F. Pan, Y. Gao, H. Li, X. Qin, Y. Jiang, J. Qi, J. Xie, S. Jia. *Analysis of components and properties of extractives from *Alnus cremastogyne* pods from different provenances*, *Molecules.* 27 (2022) 7802.
  35. M. R. Zahi, H. Liang, Q. Yuan. *Improving the antimicrobial activity of D-limonene using a novel organogel-based nanoemulsion*, *Food Con.* 50 (2015) 554-559.
  36. A. Hongsiri, S. Duanyai. *Antioxidant activity, total phenolic and essential oil from amonum bioflorum jack*, *Adv. Sci. J.* 22 (2022) R36-R47.