Research article

Development of New Functional Beverage: Longan Kefir Supplemented with Herbs

Kasirawat Sawangrat[1], Phatthanapong Therdtatha[2], Aumpaka Pundee[2], Phisit Seesuriyachan[2] and Niphawan Panti[2]*

[1]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand*
[2]*Department of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand*

Curr. Appl. Sci. Technol. 2024, Vol. 24 (No. 6), e0259608; https://doi.org/10.55003/cast.2024.259608

Received: 3 July 2023 Revised: 13 February 2024 Accepted: 27 April 2024, Published: 2 August 2024

Abstract

Keywords

water kefir;
longan;
rosella;
butterfly pea;
safflower;
fermented beverages

In recent years, the increasing awareness of diseases has led to the demand for functional foods and beverages that provide additional health benefits beyond basic nutrition. Water kefir is a healthy fermented beverage with unique aroma and flavor profile, obtained by the symbiotic fermentation of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeast. In this study, a new functional beverage based on substrates such as longan juice (KL) and longan juice supplemented with herbs: butterfly pea (KLB), rosella (KLR), or safflower (KLS), and fermented at 25°C for 48 h with water kefir grains as starter, was developed. The occurrence of microbial community of LAB, AAB and yeast during fermentation in all treatments were not significantly different ($p<0.05$) However, each supplement showed its distinct color according to specific characters of the herbs. The addition of the herbs significantly enhanced total phenolic compounds (TPCs) and antioxidant activities as investigated by DPPH and ABTS assay, with the results varying with supplement type. The addition of KLS showed the highest TPC of 212.17 µgGAE/mL, while KLB and KLR displayed lower levels of 198.36 and 201.93 µgGAE/mL, respectively. Levels of DPPH and ABTS in all supplements showed the best condition at 24 h fermentation, but decreased at 48 h, suggesting the sensitivity of some antioxidants. Sensory evaluation revealed that KLS had the highest acceptance, while KLR was the least accepted by consumers. This study pointed out that longan kefir supplemented with herbal ingredients has the potential to be a novel functional beverage.

*Corresponding author: Tel.: +66-85-614-2947
E-mail: niphawan.p@cmu.ac.th

1. Introduction

The current consumer awareness of the importance of maintaining good health and preventing disease through diet and lifestyle choices has led to the increased development of functional foods. Consumers have become more health-conscious and seek out foods that can provide additional health benefits beyond basic nutrition. These types of foods are designed to improve health and well-being by providing specific nutrients or bioactive compounds that can have a positive impact on the body's functions.

Water kefir is a healthy fermented beverage that has been consumed for centuries in various countries around the world. It is made by fermenting a mixture of sucrose solution or fruit juice with sugary kefir grains, which are transparent gelatinous structures composed of lactic acid bacteria, acetic acid bacteria, and yeast. The microorganisms in kefir grains utilize the sugar in the water, converting it into organic acid, aromatic compounds, ethanol, carbon dioxide, and amino acids which provide unique characteristics such as fizziness, slightly sweet and sour flavor. It is a good source of vitamins, minerals, and antioxidants, and is often used as a remedy beverage for a variety of ailments including constipation, diarrhea, and bloating as well as possessing anti-diabetic, anti-carcinogenic, and anti-inflammatory properties [1]. Furthermore, water kefir is an excellent source of beneficial microbes that are recognized probiotic candidates. These microbes proliferate in a healthy gut microbiome, enhancing digestion, immune function, and overall health. Water kefir is very versatile and can be flavored with a variety of supplements such as fruit, herbs, or spices that are added during the fermentation process [2-4]

Longan (*Dimocarpus longan*) is a tropical fruit tree that belongs to the family Sapindaceae. It is native to Southeast Asia and has been widely cultivated in many countries such as Thailand, Vietnam, China, India, Australia, and Philippines. Particularly in Thailand, longan is an essential cash crop grown in the northern region of the country, and 80-90% of the production is exported to China [5]. However, longan prices have dropped over the past 30 years due to overproduction and undesirable quality. Longan is rich in fiber, carbohydrates, amino acids, vitamin C, polyphenols, and minerals that promote health benefits including improved digestion, immune system support, anticancer, and cardiovascular system support [6, 7]. As mentioned, these point out an important opportunity for developing healthy products from longan with concomitant raising of its quality and value.

Butterfly pea (*Clitoria ternatea*), safflower (*Carthamus tinctorius*), and rosella (*Hibiscus sabdariffa*) are native plants in Asia that are often used as natural dyes for food and other products. Besides, these plants have been used in traditional medicines and it is believed to have potential health benefits, including lowering blood pressure and cholesterol levels, reducing inflammation, and improving digestion [8-10]. According to these properties, the development of longan water kefir beverage fortified with these herbs was our interest.

The aim of this study was to investigate the properties of longan water kefir supplemented with butterfly pea, safflower, and rosella in term of microbiological, physical, chemical, and sensory evaluation and to create a functional beverage that has the potential to provide a range of health benefits. Combination of these herbs and kefir provided a functional beverage with the potential for numerous health benefits, particularly antioxidant enhancement. By increasing antioxidant status, this fortified water kefir may contribute to the prevention of chronic diseases. This study highlights the growing importance of dietary choices in maintaining good health and preventing diseases. With continued research and development, functional foods like these have the potential to play an increasingly important role in promoting health and well-being in the years to come.

2. Materials and Methods

2.1 Materials

Water kefir grains were purchased from Nature Kombucha (Khon Kaen, Thailand, location: 16.439891, 102.829019). The propagation of water kefir grains was performed by adding the grains 5% (w/v) into 100 mL of 15% (w/v) brown sugar solution, and incubated at room temperature for 24 h. The grains were then recovered using a sieve for assay. Dried longan, butterfly pea, safflower, and rosella were purchased from local markets (Chiang Mai, Thailand, location: 18.784998, 98.984808).

2.2 Preparation of water kefir beverages with herbs

Longan juice was obtained by boiling dried longan with distilled water in a ratio of 1:10 for 10 min. For herbal beverages, each dried herb was extracted in distilled water in a ratio of 1:5 for 5 min, the beverages were then adjusted to reach 20% brix using sucrose before pasteurized at 70°C for 3 min and cooled to room temperature. To prepare longan kefir with herbs, longan juice was mixed with each herbal extract in a ratio of 1:1 (v/v), followed by adding 5% (w/v) water kefir grains to the solutions. The fermentation process was performed in a 250 mL Erlenmeyer flask, with a capacity of 100 mL substrate and incubated for 24 and 48 h, at 25°C. Water kefir samples were filtered and stored at 4°C for analysis.

2.3 Microbiological analysis

Microbiological analysis was performed using the drop plate technique as described by Reed and Reed [11]. For viable cell count, lactic acid bacteria (LAB) were grown on De Man Rogosa Sharpe (MRS) medium (Himedia, India) supplemented with cycloheximide (100 mg/mL), and incubated under anaerobic condition at 37°C for 24 h whereas acetic acid bacteria (AAB) were grown on Carr medium prepared with cycloheximide (100 mg/mL) and penicillin (100 mg/mL) as described by Klawpiyapamornkun *et al*. [12], and incubated under aerobic condition at 30°C for 24 h. Yeasts were grown on Dichloran Rose Bengal Chloramphenicol agar (Himedia, India), and incubated under aerobic condition at 25°C for 24 h. The average number of colonies was then determined and expressed as log CFU (colony forming units)/mL.

2.4 Physicochemical measurements

Color analyses were measured using a Color Quest XE spectrophotometer (Hunter Lab, USA). The colorimetric parameters obtained were L* (lightness), a* (redness), and b* (yellowness) values. These values were calculated and expressed as the total difference (ΔE*) using the equation below.

$$\Delta E^{*} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{2}} \quad (1)$$

2.5 Determination of pH

The pH values of kefir beverages were determined using an electronic pH meter (Denver Instrument UB-10, USA)

2.6 Determination of total phenolic content

The total phenolic contents of kefir beverages were determined according to the Folin-Ciocalteu method [13]. Each reaction mixture that was composed of 120 µL of sample and 600 µL 10% (v/v) Folin-Ciocalteu reagent (Sigma-Aldrich, MO, USA) was shaken and incubated at room temperature for 30 min. Absorbance was measured at 765 nm using a UV-VIS spectrophotometer (Genesys 30, Thermo Scientific, USA). TPC was calculated as GAE per gram of dry weight using a standard gallic acid curve.

2.7 Determination of DPPH radical scavenging

The free radical scavenging ability of the kefir beverages was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH assay was conducted according to Xiao *et al*. [14], with some modifications. DPPH solution was prepared by dissolving 7.89 mg DPPH with 100 mL 99.5% methanol. Then, DPPH solution (1,500 µL) was added in a test tube that contained 500 µL sample and 500 µL methanol. The solution was kept in the dark place for 20 min. The absorbance was measured at 515 nm using a UV-VIS spectrophotometer (Genesys 30, Thermo Scientific, USA). The radical scavenging activity was calculated per the following equation.

$$\% \text{ DPPH radical scavenging} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100 \quad (2)$$

2.8 Determination of ABTS radical scavenging

The antioxidant capacity of samples was determined using 2,2-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) as described by Xiao *et al*. [14]. Gallic acid was used as the standard. For each sample, a solution containing 100 µL sample and 200 µL ABTS working solution was incubated in the dark for 7 min. Then, absorbance was measured at 739 nm using a UV-VIS spectrophotometer (Genesys 30, Thermo Scientific, USA). The percentage of ABTS scavenging activity was calculated per the following formula.

$$\% \text{ ABTS radical scavenging} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100 \quad (3)$$

2.9 Determination of sugars and organic acids

Sugar (sucrose, glucose, and fructose), organic acids (lactic acid, formic acid, acetic acid, butyric acid), and alcohol were determined by HPLC (5450, Chromaster, Hitachi, Japan) equipped with a Refractive Index Detector (RID) and Aminex HPX-87P Column (7.8 x 300 mm, 10 µL), with the use of ChromAssist program (version 2.1), and 5 mM H_2SO_4 was used as mobile phase. The flow rate and temperature were maintained at 0.4 mL/min and 45°C, respectively. All samples were diluted at the optimum concentration and filtered using 0.22 µm nylon syringe filter before analysis.

2.10 Determination of titratable acidity

Titratable acidity was determined by titratable method according to AOAC [15] using 0.1 N NaOH. The result was expressed as acetic acid.

2.11 Sensory evaluation

This study was exempted from ethical approval by ethics committee of Chiang Mai University (CMUREC 66/088). The sensory evaluation was determined by 50 untrained-panelists (15-44 years old) consisting of males (n=12) and females (n=38). All participants were recruited randomly in Suthep (location: 18.766998, 98.941492) and Mae Hia (location: 18.754391254767302, 98.96862554139709) subdistricts in Chiang Mai province. Water kefir beverages were stored at 4°C before the evaluation. All participants were asked to first drink water to clean their mouths. The 9-point hedonic scale (9 = extremely pleasant; 1 = extremely unpleasant) was used to evaluate the properties of beverages.

2.12 Statistical analysis

The experimental design in this study used a Completely Randomized Design (CRD). The treatment design consisted of two factors, which were the type of herb (rosella, safflower, and butterfly pea) and the duration of fermentation time (24 and 48 h). Microbiological parameters (LAB, AAB, and yeast), color attributes, chemical properties (pH, TPC, DPPH, ABTS, sugars, and organic acids), and sensory characteristics underwent exploratory multivariate analysis to examine the relationships among the data collected from various experiments. All data were shown as the mean±standard deviation (SD) of three replicates. ANOVA was performed to evaluate the statistical significance of the differences suggested by Duncan's ($p<0.05$) using SPSS (version 29.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Microbiological properties

In this study, water kefir grains were cultivated on different substrates consisting of longan juice mixed with each herbal extract, which was rosella (KLR), safflower (KLS), or butterfly pea (KLB), and longan juice (KL) was used as a control. All samples were incubated at 25°C for 48 h. A study was conducted to investigate the impact of fermentation time and type of substrate on the growth of three groups of microorganisms (LAB, AAB, and yeast). The results indicated that all three groups of microorganisms exhibited a significant increase in growth with increase in fermentation time. However, the type of substrate used was found to have a significant impact on the growth of yeast, with KLR and KLB substrates demonstrating slower growth rates due to some bioactive components of rosella and butterfly pea having inhibitory effects against some types of yeasts [16-19].

As shown in Figure 1 (a-c), throughout the fermentation period, acetic acid bacteria were found to predominant in all kefir samples. By the end of fermentation, the viable cell number of acetic acid bacteria varied between 8.18-8.35 log CFU/mL, following by lactic acid bacteria at 7.91-8.18 log CFU/mL, and yeast at 6.16-6.52 log CFU/mL. These results demonstrated the similar trend of the study on water kefir microbial diversity reported by Gulitz *et al*. [20]. It is plausible that the longan juices, known for their high sugar content, and incubation at the optimal yeast growth temperature, are likely to promote the proliferation of yeast, thereby facilitating alcohol production and subsequent growth of acetic acid bacteria (AAB). Additionally, it is reasonable to assume that kefir grains used in this study may contain higher numbers of AAB, leading to an increased presence of AAB compared to lactic acid bacteria (LAB).

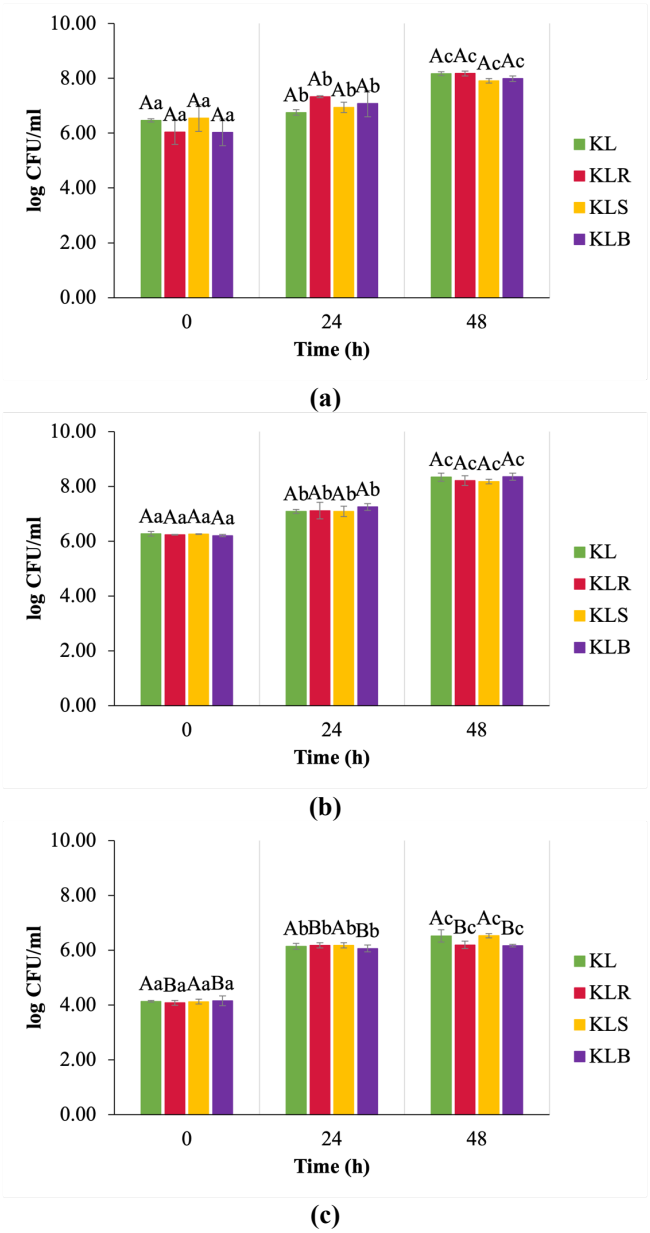


Figure 1. The microbial count (log CFU/mL) for lactic acid bacteria (a), acetic acid bacteria (b), and yeast (c) during the 48-h fermentation of different water kefir beverages. The data represents the mean values of triplicate determinations. Different superscript capital letters and small letters represent significantly different values of differences in substrate types and fermentation times, respectively ($p<0.05$).

3.2 Color and pH

As shown in Figure 2 and Table 1, the addition of herbs and the duration of fermentation significantly impacted the color of kefir samples. In particular, the color difference (ΔE*) values of water kefir with KLR and KLB demonstrated distinct variations across fermentation period, and the alterations could likely be attributed to the presence of anthocyanins in rosella and butterfly pea changing chemical structure with pH. The anthocyanins provide a range of colors from salmon-pink and red to violet and blue with decrease of pH during fermentation [21-25].

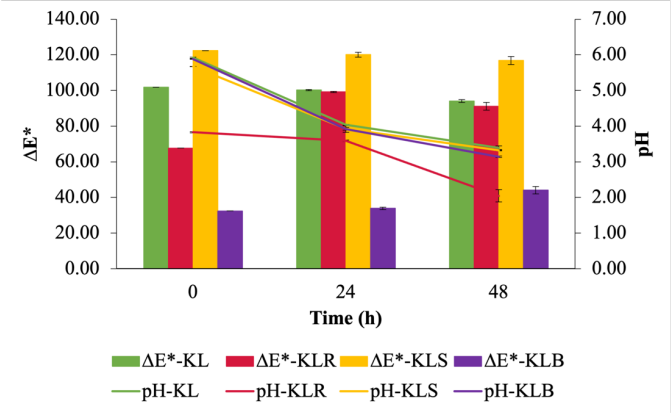


Figure 2. Color difference (ΔE*) values and pH changes of water kefir beverages

Table 1. The chromatic parameters of water kefir beverages

Types	Time	Colorimeter		
		Lightness (L*)	Redness (a*)	Yellowness (b*)
KL	0	85.02±0.00^{Aa}	5.50±0.00^{Aa}	55.61±0.00^{Ba}
	24	84.21±0.87 ^{Aa}	5.71±0.70^{Aa}	54.07±1.61^{Ba}
	48	83.41±1.64^{Aa}	3.82±0.50^{Aa}	43.40±1.45^{Ba}
KLR	0	59.24±0.00^{Ba}	31.73±0.00^{Ba}	8.11±0.00^{Ba}
	24	75.33±2.24^{Bb}	16.82±1.94^{Bb}	62.14±2.55^{Bb}
	48	62.70±2.55^{Bb}	22.04±0.90^{Bb}	62.35±0.80^{Bb}
KLS	0	67.68±0.00^{Ba}	20.72±0.00^{Ba}	99.75±0.00^{Aa}
	24	68.84±3.65^{Bb}	18.99±3.81^{Ba}	96.44±0.56^{Aa}
	48	64.92±1.85^{Bb}	20.19±0.70^{Ba}	94.98±1.79^{Aa}
KLB	0	14.25±0.00^{Ca}	22.53±0.00^{Ba}	-18.40±0.00^{Ca}
	24	21.94±0.85^{Cb}	25.68±1.42^{Bb}	0.32±1.89^{Cb}
	48	31.37±6.53^{Cb}	29.24±3.47^{Bb}	8.12±2.58^{Cb}

Data represents the mean values of triplicate determinations±standard deviation. Different superscript capital letters in the same column and small letters in the same row indicate significantly different values of differences in substrate types and fermentation times, respectively ($p < 0.05$).

3.3 Total phenolic content and antioxidant activity

The effects of fermentation time on the total phenolic content (TPC) and antioxidant activity of longan kefir beverages with added herbs were investigated. The addition of herbs led to a significant increase in TPC, with the KLS sample showing the highest TPC value (189.07-212.17 µg GAE/mL), followed by KLB (169.07-198.36 µg GAE/mL), KLR (161.21-201.93 µg GAE/mL), and KL (84.07-168.83 µg GAE/mL), respectively. Interestingly, the impact of fermentation time on TPC was variable among the samples. KL and KLB displayed an initial increase in TPC at 24 h, followed by a rapid decrease. In contrast, KLR and KLS demonstrated a gradual increase in TPC at each 24-h interval, as illustrated in Figure 3. It was plausible that KLR and KLS might contain additional compounds or bioactive components that contributed to an increase in total phenolic compound content during fermentation. These added ingredients could potentially have caused the release or enhancement of the formation of phenolic compounds during the fermentation process, leading to an increase in total phenolic content [26-33]. Furthermore, microorganisms could interact with the phenolic compounds present in the ingredients, leading to the conversion or degradation of certain phenolic compounds. The specific microorganisms present in the fermentation process and their metabolic activities could vary depending on the ingredients used [34]. It is possible that the microorganisms present during the fermentation of KL and KLB might have a higher tendency to metabolize or degrade phenolic compounds, resulting in a decrease in total phenolic content over time [35, 36].

The antioxidant activity of the water kefir beverages was evaluated using the DPPH and ABTS assays. The results indicated that water kefir with KLR had the highest antioxidant activity (80.44%) as measured by the DPPH assay, followed by KLS (65.64%), KLB (60.36%), and KL (50.26%) at 24 h of fermentation (Figure 4). However, a significant decrease in antioxidant activity was observed with an increase in fermentation time, as was seen with the ABTS assay. Interestingly, most of the unfermented samples (at 0 h) displayed the highest antioxidant activities, including KLR (84.97%), KLC (66.60%), and KL (66.45%), except for KLS, which exhibited the highest activity at 24 h (81.96%) (Figure 5). The findings of this study suggested that the addition of herbs can enhance the antioxidant activity of water kefir beverages. However, the impact of fermentation time on antioxidant activity was variable among the samples. The decrease in antioxidant activity observed may be due to factors like the sensitivity of some antioxidants including vitamin C and polyphenols to light during the fermentation process [37, 38], the interaction of reactive oxygen species (ROS) with antioxidants produced during microbial metabolism [39, 40], and the production of organic acids leading to a decrease in pH that affected the stability of antioxidants [41].

3.4 Sugars analysis

The sugar analysis results (sucrose, glucose, and fructose) are shown in Table 2. The unfermented kefir samples had sucrose as the predominant sugar [42], ranging from 13.76-14.81 mg/mL, which was attributed to the addition of brown sugar and longan juice. Glucose levels ranged from 0.44-0.64 mg/mL, while fructose levels ranged from 0.39-0.60 mg/mL. After fermentation, the sucrose content decreased significantly while glucose and fructose levels slightly increased. This could be due to the action of invertase enzymes produced by microorganisms such as *Saccharomyces cerevisiae*, *Arctocephalus tropicalis*, *Amycolatopsis orientalis* and *Pichia membranifaciens* catalyzing the breakdown of sucrose into glucose and fructose, which are more readily absorbed by other microorganisms in water kefir [43]. Interestingly, the KLR samples exhibited the highest sucrose utilization activity, resulting in higher levels of glucose and fructose compared to other kefir samples. This might be due to the presence of some bioactive compounds in rosella that promote

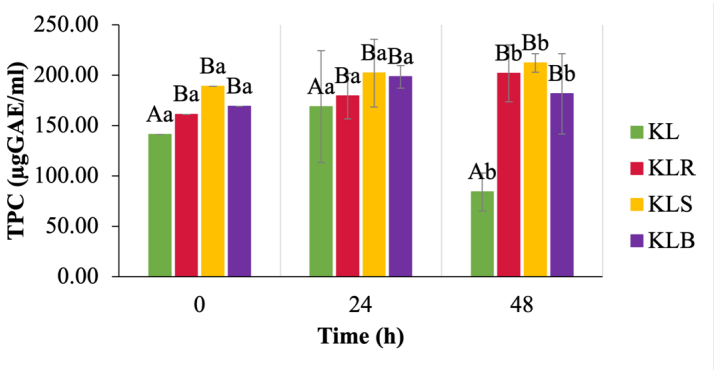


Figure 3. The total phenolic compound content changes of water kefir beverages. Different superscript capital letters and small letters represent significantly different values of differences in substrate types and fermentation times, respectively ($p<0.05$).

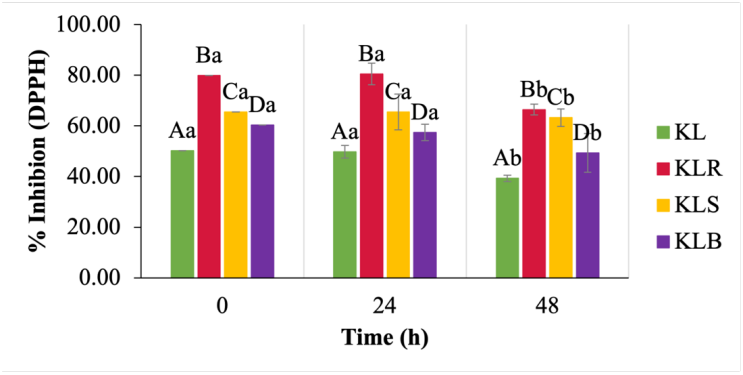


Figure 4. DPPH radical scavenging activity (%) changes of water kefir beverages. Different superscript capital letters and small letters represent significantly different values of differences in substrate types and fermentation times, respectively ($p<0.05$).

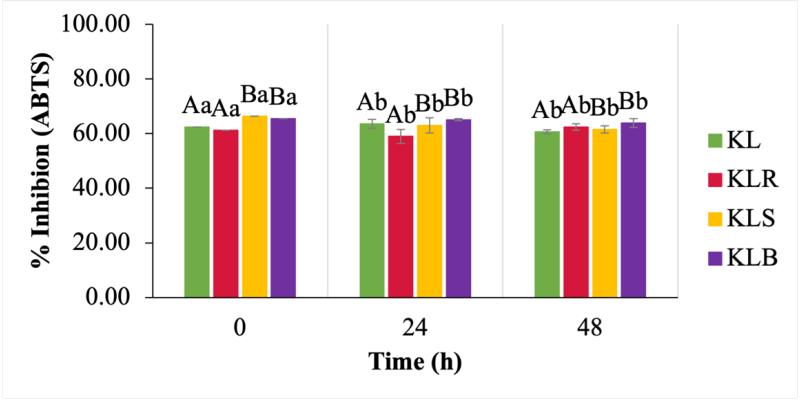


Figure 5. ABTS radical scavenging activity (%) changes of water kefir beverages. Different superscript capital letters and small letters represent significantly different values of differences in substrate types and fermentation times, respectively ($p < 0.05$).

Table 2. Changes in the sugar content of water kefir beverages during fermentation

Types	Time	Sugar Content (mg/mL)		
		Sucrose	Fructose	Glucose
KL	0	13.76 ± 0.00^{Aa}	0.46 ± 0.00^{Aa}	0.43 ± 0.00^{Aa}
	24	9.61 ± 0.95^{Ab}	1.21 ± 0.05^{Ab}	1.31 ± 0.05^{Ab}
	48	4.58 ± 0.55^{Ac}	0.84 ± 0.13^{Ab}	1.06 ± 0.12^{Ac}
KLR	0	14.62 ± 0.00^{Ba}	0.64 ± 0.00^{Ba}	0.60 ± 0.00^{Ba}
	24	5.15 ± 0.36^{Bb}	3.48 ± 0.32^{Bb}	3.30 ± 0.30^{Bb}
	48	3.19 ± 0.48^{Bc}	4.17 ± 0.42^{Bb}	4.22 ± 0.47^{Bc}
KLS	0	14.81 ± 0.00^{Aa}	0.45 ± 0.00^{Aa}	0.42 ± 0.00^{Aa}
	24	7.44 ± 0.15^{Ab}	1.09 ± 0.15^{Ab}	1.19 ± 0.15^{Ab}
	48	7.28 ± 0.55^{Ac}	1.54 ± 0.22^{Ab}	1.72 ± 0.08^{Ac}
KLB	0	14.57 ± 0.00^{Aa}	0.44 ± 0.00^{Ca}	0.39 ± 0.00^{Ca}
	24	10.53 ± 1.72^{Ab}	1.82 ± 0.60^{Cb}	1.80 ± 0.51^{Cb}
	48	6.55 ± 1.98^{Ac}	1.93 ± 0.40^{Cb}	2.10 ± 0.47^{Cc}

Data are mean values of triplicate determinations±standard deviation. Different superscript capital letters in the same column and small letters in the same row indicate significantly different values of differences in substrate types and fermentation times, respectively ($p<0.05$).

the growth of *Lactobacillus* species that could efficiently utilize sucrose, as shown in Figure 1a. It is also likely that *Lactobacillus hilgardii*, which is commonly found in water kefir. This species is usually responsible for sucrose utilization for the formation of dextran in water kefir grain formation [44].

3.5 Organic acid analysis

The predominant organic acids found during fermentation were lactic acid and acetic acid, as shown in Table 3. Among the four variations, the KL and KLS samples exhibited a higher concentration of lactic acid at the end of fermentation, while acetic acid was the main organic acid observed in the KLR sample. Conversely, in the case of the KLB, both lactic acid and acetic acid were detected at similar levels. These results suggested that each substrate may contain specific compounds or nutrients that stimulate the growth of specific microbial groups, leading to the production of lactic acid or acetic acid. Safflower and butterfly pea contain various bioactive compounds such as polyphenols, flavonoids, and terpenoids that have prebiotic properties which be able to stimulate the growth of lactic acid bacteria, and hence are known as probiotics [45, 46]. On the other hand, the organic acids in rosella such as hibiscus acid, citric acid, hydroxycitric acid, malic acid, tartaric acid, and ascorbic acid tend to produce an acidic pH environment that contributes to the growth of acetic acid bacteria [47]. However, further research is required to fully understand the factors influencing the metabolism of these microorganisms in each substrate. Furthermore, the overall organic acid contents of each water kefir were positively correlated to titratable acid results (Figure 6).

Table 3. Chang in the organic acid content water kefir beverages during fermentation

Types	Time	Organic Acid Content (mg/mL)			
		Lactic Acid	Formic Acid	Acetic Acid	Butyric Acid
KL	0	0.04±0.00^{Aa}	n.d.	n.d.	0.02±0.00^{Aa}
	24	0.27±0.07^{Aa}	0.05±0.01^{Aa}	0.05±0.01^{Aa}	0.11±0.02^{Aba}
	48	0.28±0.07^{Ab}	0.10±0.04^{Ab}	0.16±0.03^{Ab}	0.19±0.11^{Abb}
KLR	0	n.d.	n.d.	n.d.	n.d.
	24	0.09±0.05^{Ba}	0.05±0.01^{Aa}	0.04±0.01^{Ba}	0.09±0.02^{Aa}
	48	0.14±0.04^{Bb}	0.10±0.02^{Ab}	0.33±0.04^{Bb}	0.12±0.05^{Ab}
KLS	0	0.03±0.00^{Aa}	n.d.	n.d.	0.03±0.00^{Aa}
	24	0.15±0.03^{Aa}	0.06±0.02^{Aa}	0.06±0.05^{Aa}	0.14±0.03^{Ba}
	48	0.33±0.15^{Ab}	0.11±0.04^{Ab}	0.17±0.09^{Ab}	0.28±0.10^{Bb}
KLB	0	0.03±0.00^{Aa}	n.d.	n.d.	0.02±0.00^{Aa}
	24	0.24±0.02^{Aa}	0.05±0.02^{Aa}	0.07±0.01^{Ba}	0.11±0.05^{Aa}
	48	0.39±0.19^{Ab}	0.08±0.02^{Ab}	0.34±0.12^{Bb}	0.12±0.04^{Ab}

Data are mean values of triplicate determinations ± standard deviation. Different superscript capital letters in the same column and small letters in the same row indicate significantly different values of differences type of substrates and fermentation times, respectively ($p<0.05$). n.d. presented not detected.

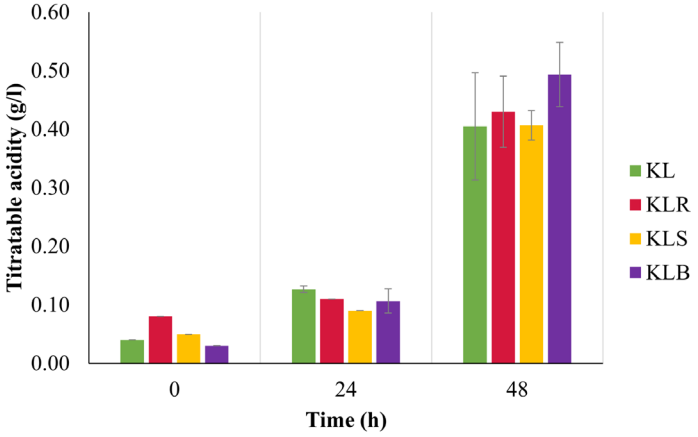
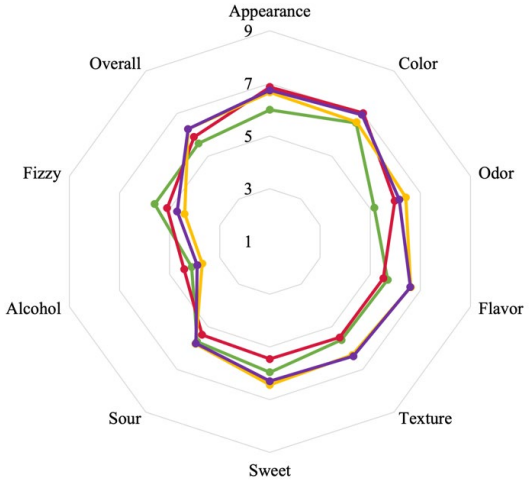


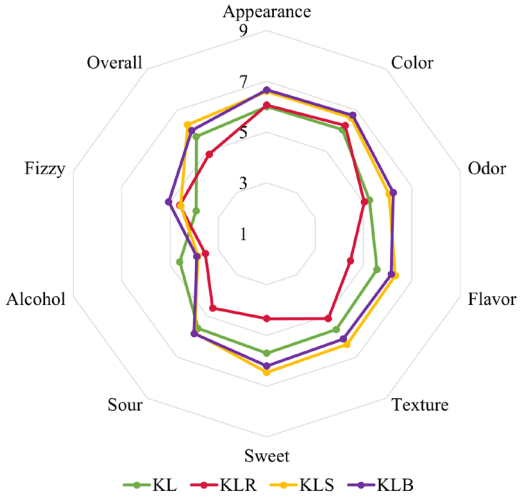
Figure 6. Changes in the total acidity (%) of the water kefir beverages

3.6 Sensory analysis

The sensory properties of different water kefir beverages were evaluated using a 9-point hedonic scale that assessed various attributes including appearance, color, odor, flavor, texture, sweetness, sourness, alcohol content, fizziness, and overall acceptance. A total of 50 untrained testers participated in the study, and the results are reported in Figure 7(a, b). In the statistical analysis of



(a)



(b)

Figure 7. Sensory properties evaluation on water kefir beverages fermented for 24 h (a) and 48 h (b)

the effects of herb types on the sensory evaluation of longan kefir, it was observed that KLR had the lowest score, except for appearance. In contrast, KLS and KLB received significantly higher scores for flavor, sweetness, and overall satisfaction. This difference might be attributed to the addition of safflower and butterfly pea, as they do not notably affect the pH of the beverages and they also possess mild flavors that do not disturb the characteristics of longan kefir. In contrast, rosella imparts flavors and aromas, contributing to the acidity of the beverage (Figure 2), resulting in KLR receiving the lowest score. Regarding the relationship between fermentation time and type of herbs, significant changes were observed in the scores for flavor, sweetness, sourness, and overall satisfaction. These results indicate that extended fermentation time led to an increase in acidity and

potential alterations in odors produced by microorganisms. Consequently, the product became more acidic, resulting in a decrease in acceptance scores across various aspects. Among the different water kefir beverages evaluated, KLS showed the highest overall acceptance, followed by KLB, KLR, and KL. These findings suggest that the addition of herbs improved the preference for longan kefir, possibly as they supplied unique color, flavors, and aromas to the beverage.

4. Conclusions

This study investigated the effects of adding herbal extracts to longan water kefir. The results demonstrated that the microbial diversity of longan water kefir was dominated by AAB, followed by LAB and yeast, respectively. Furthermore, the addition of herbs to the fermentation process resulted in a notable increase in total phenolic content, antioxidant activity, and sensory evaluation. These novel fermented products have the potential to serve as a valuable alternative source of beneficial microorganisms for those who are interested in functional foods, vegetarians, and consumers who have allergies to dairy products.

5. Acknowledgements

The authors would like to express their gratitude to the Faculty of Agro-Industry, Chiang Mai University for the funding support.

References

[1] Guzel-Seydim, Z.B., Gökırmaklı, Ç. and Greene, A.K., 2021. A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. *Trends in Food Science and Technology*, 113, 42-53.

[2] Moretti, A.F., Moure, M.C., Quiñoy, F., Esposito, F., Simonelli, N., Medrano, M. and León-Peláez, Á., 2022. Water kefir, a fermented beverage containing probiotic microorganisms: From ancient and artisanal manufacture to industrialized and regulated commercialization. *Future Foods*, 5, https://doi.org/10.1016/j.fufo.2022.100123.

[3] Lynch, K.M., Wilkinson, S., Daenen, L. and Arendt, E.K., 2021. An update on water kefir: Microbiology, composition and production. *International Journal of Food Microbiology*, 345, https://doi.org/10.1016/j.ijfoodmicro.2021.109128.

[4] Cai, Y., Sounderrajan, A. and Serventi, L., 2020. Water kefir: A review of its microbiological profile, antioxidant potential and sensory quality. *Acta Scientific Nutritional Health*, 4(6), 10-17.

[5] Shao, N., 2022. Riding on waves and filling the cracks: Toward an inclusive and sustainable Sino-Thai longan supply chain management in upper Thailand. *ASR Chiang Mai University Journal of Social Sciences and Humanities*, 9(2), 1-17.

[6] Zhang, X., Guo, S., Ho, C.-T. and Bai, N., 2020. Phytochemical constituents and biological activities of longan (*Dimocarpus longan* Lour.) fruit: A review. *Food Science and Human Wellness*, 9(2), 95-102.

[7] Yang, B., Jiang, Y., Shi, J., Chen, F. and Ashraf, M., 2011. Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour.) fruit—A review. *Food Research International*, 44(7), 1837-1842.

[8] Lijon, M.B., Meghla, N.S., Jahedi, E., Rahman, M.A. and Hossain, I., 2017. Phytochemistry and pharmacological activities of *Clitoria ternatea*. *International Journal of Natural and Social Sciences*, 4(1), 1-10.

[9] Zhang, L.-L., Tian, K., Tang, Z.-H., Chen, X.-J., Bian, Z.-X., Wang, Y.-T. and Lu, J.-J., 2016. Phytochemistry and pharmacology of *Carthamus tinctorius* L. *The American Journal of Chinese Medicine*, 44(02), 197-226.

[10] Riaz, G. and Chopra, R., 2018. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomedicine and Pharmacotherapy*, 102, 575-586.

[11] Reed, R.W. and Reed, G.B., 1948. "Drop plate" method of counting viable bacteria. *Canadian Journal of Research*, 26(6), 317-326.

[12] Klawpiyapamornkun, T., Bovonsombut, S. and Bovonsombut, S., 2015. Isolation and characterization of acetic acid bacteria from fruits and fermented fruit juices for vinegar production. *Food and Applied Bioscience Journal*, 3(1), 30-38.

[13] Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

[14] Xiao, F., Xu, T., Lu, B. and Liu, R., 2020. Guidelines for antioxidant assays for food components. *Food Frontiers*, 1(1), 60-69.

[15] AOAC, 2003. *Official Method of Analysis*. 17th Edition. Washington, D.C.: Association of Analytical.

[16] Hamrita, B., Emira, N., Papetti, A., Badraoui, R., Bouslama, L., Ben Tekfa, M. I., Hamdi, A., Patel, M., Elasbali, A.M., Adnan, M., Sahraf, S.A. and Snoussi, M., 2022. Phytochemical analysis, antioxidant, antimicrobial, and anti-swarming properties of *Hibiscus sabdariffa* L. calyx extracts: *In vitro* and *in silico* modelling approaches. *Evidence-Based Complementary and Alternative Medicine*, 2022, https://doi.org/10.1155/2022/1252672.

[17] Çömlekcioğlu, N. and Aygan, A., 2020. Fatty acids, bioactive content and antimicrobial activity of *Hibiscus sabdariffa* L. extract obtained by different techniques. *Turkish Journal of Agriculture-Food Science and Technology*, 8(12), 2723-2728.

[18] Deorankar, P., Gangiwale, R., Chintamani, R. and Singh, R.P., 2020. Evaluation of ethanolic and aqueous extract of *Clitoria ternatea* for antimicrobial activity. *Indian Journal of Natural Products and Resources*, 11(3), 194-198.

[19] Jeyaraj, E.J., Lim, Y.Y. and Choo, W.S., 2021. Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals. *Journal of Food Science and Technology*, 58(6), 2054-2067.

[20] Gulitz, A., Stadie, J., Wenning, M., Ehrmann, M.A. and Vogel, R.F., 2011. The microbial diversity of water kefir. *International Journal of Food Microbiology*, 151(3), 284-288.

[21] Roy, S. and Rhim, J.W., 2021. Anthocyanin food colorant and its application in pH-responsive color change indicator films. *Critical Reviews in Food Science and Nutrition*, 61(14), 2297-2325.

[22] Martínez, A., Vegara, S., Herranz-López, M., Martí, N., Valero, M., Micol, V. and Saura, D., 2017. Kinetic changes of polyphenols, anthocyanins and antioxidant capacity in forced aged hibiscus ale beer. *Journal of the Institute of Brewing*, 123(1), 58-65.

[23] Amperawati, S., Hastuti, P., Pranoto, Y. and Santoso, U., 2019. The anthocyanins content, color changes and thermal stability of roselle (*Hibiscus sabdariffa* L.) petal extract. *International Journal of Science and Research*, 8(4), 428-435.

[24] Kim, H.-J., Roy, S. and Rhim, J.-W., 2022. Gelatin/agar-based color-indicator film integrated with *Clitoria ternatea* flower anthocyanin and zinc oxide nanoparticles for monitoring freshness of shrimp. *Food Hydrocolloids*, 124, https://doi.org/10.1016/j.foodhyd.2021.107294.

[25] Kungsuwan, K., Singh, K., Phetkao, S. and Utama-ang, N., 2014. Effects of pH and anthocyanin concentration on color and antioxidant activity of *Clitoria ternatea* extract. *Food and Applied Bioscience Journal*, 2(1), 31-46.

[26] Salem, N., Msaada, K., Hamdaoui, G., Limam, F. and Marzouk, B., 2011. Variation in phenolic composition and antioxidant activity during flower development of safflower (*Carthamus tinctorius* L.). *Journal of Agricultural and Food Chemistry*, 59(9), 4455-4463.

[27] Jung, Y.J., Assefa, A.D., Lee, J.E., Lee, H.S., Rhee, J.H. and Sung, J.S., 2019. Analysis of antioxidant activity and serotonin derivatives in safflower (*Carthamus tinctorius* L.) germplasm collected from five countries. *Korean Journal of Plant Resources*, 32(5), 423-432.

[28] Buyukkurt, O.K., Guclu, G., Barutcular, C., Selli, S. and Kelebek, H., 2021. LC-MS/MS fingerprint and simultaneous quantification of bioactive compounds in safflower petals (*Carthamus tinctorius* L.). *Microchemical Journal*, 171, https://doi.org/10.1016/j.microc.2021.106850.

[29] Karimkhani, M.M., Shaddel, R., Khodaparast, M.H.H., Vazirian, M. and Piri-Gheshlaghi, S., 2016. Antioxidant and antibacterial activity of safflower (*Carthamus tinctorius* L.) extract from four different cultivars. *Quality Assurance and Safety of Crops and Foods*, 8(4), 565-574.

[30] Borrás-Linares, I., Fernández-Arroyo, S., Arráez-Roman, D., Palmeros-Suárez, P.A., Del Val-Díaz, R., Andrade-Gonzáles, I., Fernández-Gutiérrez, A., Gómez-Leyva, J.F. and Segura-Carretero, A., 2015. Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican roselle (*Hibiscus sabdariffa*). *Industrial Crops and Products*, 69, 385-394.

[31] Lyu, J.I., Kim, J.M., Kim, D.G., Kim, J.B., Kim, S.H., Ahn, J.W., Kang, S.Y., Ryu, J. and Kwon, S.J., 2020. Phenolic compound content of leaf extracts from different roselle (*Hibiscus sabdariffa*) accessions. *Plant Breeding and Biotechnology*, 8(1), 1-10.

[32] Tsai, P.-J., McIntosh, J., Pearce, P., Camden, B. and Jordan, B.R., 2002. Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. *Food Research International*, 35(4), 351-356.

[33] Escobar-Ortiz, A., Castaño-Tostado, E., Rocha-Guzmán, N.E., Gallegos-Infante, J.A. and Reynoso-Camacho, R., 2021. Anthocyanins extraction from *Hibiscus sabdariffa* and identification of phenolic compounds associated with their stability. *Journal of the Science of Food and Agriculture*, 101(1), 110-119.

[34] Sabokbar, N. and Khodaiyan, F., 2016. Total phenolic content and antioxidant activities of pomegranate juice and whey based novel beverage fermented by kefir grains. *Journal of Food Science and Technology*, 53, 739-747.

[35] Hampton, J., Tang, C., Subhash, A.J. and Serventi, L., 2021. Assessment of pear juice and puree as a fermentation matrix for water kefir. *Journal of Food Processing and Preservation*, 45(3), https://doi.org/10.1111/jfpp.15223.

[36] Rodriguez, H., Curiel, J.A., Landete, J.M., de las Rivas, B., de Felipe, F., Gomez-Cordoves, C., Mancheño, J.M. and Muñoz, R., 2009. Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology*, 132(2-3), 79-90.

[37] Csapó, J., Prokisch, J., Albert, C. and Sipos, P., 2019. Effect of UV light on food quality and safety. *Acta Universitatis Sapientiae, Alimentaria*, 12(1), 21-41.

[38] Teixeira, C.C.C., de Freitas Cabral, T.P., Tacon, L.A., Villardi, I.L., Lanchote, A.D. and de Freitas, L.A.P., 2017. Solid state stability of polyphenols from a plant extract after fluid bed atmospheric spray-freeze-drying. *Powder Technology*, 319, 494-504.

[39] Lu, Z. and Imlay, J.A., 2021. When anaerobes encounter oxygen: mechanisms of oxygen toxicity, tolerance and defence. *Nature Reviews Microbiology*, 19(12), 774-785.

[40] Rani, A., Saini, K.C., Bast, F., Mehariya, S., Bhatia, S.K., Lavecchia, R. and Zuorro, A., 2021. Microorganisms: A potential source of bioactive molecules for antioxidant applications. *Molecules*, 26(4), https://doi.org/10.3390/molecules26041142.

[41] Bayliak, M.M., Burdyliuk, N.I. and Lushchak, V.I., 2016. Effects of pH on antioxidant and prooxidant properties of common medicinal herbs. *Open Life Sciences*, 11(1), 298-307.

[42] Surin, S., Thakeow, P., Seesuriyachan, P., Angeli, S. and Phimolsiripol, Y., 2014. Effect of extraction and concentration processes on properties of longan syrup. *Journal of Food Science and Technology*, 51(9), 2062-2069.

[43] Laureys, D., Leroy, F., Hauffman, T., Raes, M., Aerts, M., Vandamme, P. and De Vuyst, L., 2021. The type and concentration of inoculum and substrate as well as the presence of oxygen impact the water kefir fermentation process. *Frontiers in Microbiology*, 12, https://doi.org/10.3389/fmicb.2021.628599.

[44] Verce, M., De Vuyst, L. and Weckx, S., 2019. Shotgun metagenomics of a water kefir fermentation ecosystem reveals a novel *Oenococcus* species. *Frontiers in Microbiology*, 10, https://doi.org/10.3389/fmicb.2019.00479.

[45] Indriati, S., Yusuf, M., Riskayanti, R., Amaliah, N., Latief, M., Sjafruddin, R. and Attahmid, N.F.U., 2021. GC-MS and antioxidant capacity analysis in propanol extract of *Carthamus tinctorious* L. *INTEK: Jurnal Penelitian*, 8(1), 67-73.

[46] Anggraini, T., Roza, W.W., Sayuti, K. and Asben, N.A., 2022. Potential *Clitoria ternatea* as colourant for Gambir leaves tea: the antioxidant activity, polyphenols, anthocyanins, catechin, and epigallocatechin gallate. *International Journal on Advanced Science, Engineering, and Information Technology*, 12(1), 379-384.

[47] Izquierdo-Vega, J.A., Arteaga-Badillo, D.A., Sánchez-Gutiérrez, M., Morales-González, J.A., Vargas-Mendoza, N., Gómez-Aldapa, C.A., Castro-Rosas, J., Delgado-Olivares, L., Madrigal-Bujaidar, E. and Madrigal-Santillán, E., 2020. Organic acids from roselle (*Hibiscus sabdariffa* L.)- A brief review of its pharmacological effects. *Biomedicines*, 8(5), https://doi.org/10.3390/biomedicines8050100.