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Antimicrobial activity and antioxidant changes of spontaneously fermented mangosteen (*Garcinia mangostana L.*) peel

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Abstract

Mangosteen peels have high total phenolic compounds that may be enhanced through fermentation. This research aimed to study the physical, microbial analyses and antimicrobial activity of spontaneously fermented mangosteen peel (SMP). Mangosteen peels, brown sugar and water were mixed at a ratio of 1:1:10 (mangosteen peel:sugar: water) and were spontaneously fermented for 4 weeks. By the end of fermentation, the pH gradually decreased from 4.31 to 3.75 and lactic acid bacteria were significantly higher than foodborne pathogens. However, the antioxidant properties of SMP decreased by Week 4. Therefore, unfermented mangosteen peel had better performance than SMP at Week 4 for the inhibition zone, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and killing time curve assay. With the exception to *Clostridium perfringens* and *Vibrio parahaemolyticus*, SMP had an inhibition zone of 10.67 to 12.67 mm for all bacteria with no significant difference between Week 0 and Week 4. The time-kill curve of SMP at higher concentrations killed *Listeria monocytogenes* and *V. parahaemolyticus* within 1 to 4 hrs. The results showed that the unfermented mangosteen peel had better antioxidant and antimicrobial properties and had the potential as an antimicrobial agent or preservative in food.

1. Introduction

Annually, humans produce around 998 million tons of agro waste. Malaysia disposes of 168 million tons of agricultural waste (Goh, 2018), where mangosteen (*Garcinia mangostana*) peel is one of the contributors (Agamuthu, 2009). According to the Department of Agriculture Putrajaya, Malaysia produced 22,784.1 metric tons of mangosteen locally in 2015, which generated approximately 13,670 metric tons of peel waste. Mangosteen comes from the Guttiferae family and is known as the "queen of fruit" because of its unique sour taste (Midin *et al.*, 2017). The round fruit is dark purple to red-purple and consists of 30% pulp and 70% of seeds and peels. Mangosteen peel is 6 to 10 mm thick and has a hard outer layer and soft inner texture.

Several studies found that fruit peels had higher phenolic content than pulp and seed (Sadef *et al.*, 2022). A study by Wonghirundecha and Sumpavapol (2012) and Cunha *et al.* (2014) showed that mangosteen peel had the largest inhibition zone against *Bacillus cereus, Listeria monocytogenes* and *Staphylococcus aureus* as compared to rambutan peels, palmyra peels, pomegranate peels and mangosteen leaves. These antimicrobial effects are mostly associated with xanthones, a polyphenol compound in mangosteen peel with anti-inflammation, anticancer, antibacterial, antifungal (Kaomongkolgit *et al*., 2009) and antioxidant properties (Jindarat, 2009). Hien *et al.* (2019) reported that mangosteen peel from four different regions in Vietnam has 20-24% of total polyphenol content. Besides, mangosteen peel contains tannins, anthocyanins, flavonoids and other bioactive compounds (Rohman *et al.*, 2019).

Fermentation is a biodegradable process by microorganisms that breaks down big molecules and is considered a traditional food preservation method. Organic acid and other compounds produced during fermentation namely organic acids, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins may inhibit pathogen growth (Liu *et al.*, 2011) and prolong the shelf life. Apart from enhancing the flavour, fermentation increases the nutritional quality and digestibility of the food, as well as decreases the anti-nutrient compounds and toxins in food. Additionally, lactic acid bacteria

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(LAB) in fermented food showed antimicrobial properties toward food-borne pathogens (Chelule *et al.*, 2012). LAB are probiotic microorganisms that compete with other bacteria in the surroundings for nutritional requirements because they excrete mainly hydrogen peroxide, lactic acid, small proteins called bacteriocins and antibacterial peptides. For example, Lactobacillus species protect the gastrointestinal systems of humans and animals from various pathogenic bacteria (Gutiérrez *et al.*, 2016). Fermentation also increases the bioactive phenolic compound, vitamin C, and total flavonoid content, thus enhancing antioxidant activity by converting complex compounds into simpler compounds that are ready to be assimilated by the body (Hur *et al.*, 2014).

Several researchers studied on fermentation of various fruit peels. Among them was the fermentation of mango, apple, pear and banana peels for alcohol production (Reddy *et al.*, 2011; Singh *et al.*, 2014; Raganati *et al.*, 2016), cellulose production from fermented pineapple, orange and banana peel (Kannahi and Elangeswari, 2015) and fermentation of mango, banana and orange peel for citric acid production (Iralapati and Kummari, 2014). Arekemase *et al.* (2020) isolated *Lactobacillus plantarum* and *Lactobacillus casei* from rotten bananas to ferment peels of mango, banana and orange to produce lactic acid. Mango peels produced the highest amount of lactic acid (27.10%) at pH 6.0 with 2 g of substrate and 1 mL inoculum within six days with *L. casei* as the starting culture. Other than that, fermented apple by-products (a mixture of peel and pulp) had higher hydration properties, total dietary fiber and insoluble dietary fiber than raw apple by-products and chemically acidified apple by-products. The fermented apple by-products incorporated in wheat bread showed decreased bread hydrolysis index, delayed mould contamination and firming in fortified wheat bread (Cantatero *et al.*, 2019). Chanprasartsuk *et al.* (2010) also analyzed chemical changes during the fermentation of pineapple peels and the population of yeasts. These previous researchers focused only on the physicochemical changes, and acid and alcohol production of the fermented fruit peels, but did not study the antimicrobial and antioxidant properties.

The antimicrobial and antioxidant properties of fermented fruit peels are crucial to be explored because they will provide an overview of the safety and effectiveness against pathogens. Spontaneous fermentation involving microorganisms present in the natural environment was chosen because the natural microorganisms on the mangosteen peel may give a better outcome on the antimicrobial activities. Therefore, it is of interest to determine whether spontaneous fermentation may enhance the functional and antimicrobial properties of mangosteen peel. We hypothesized that the spontaneous fermentation of mangosteen peels would change the physical properties, increase the LAB count, increase the antioxidant and enhance the ability of spontaneously fermented mangosteen peels (SMP) to inhibit pathogens. Hence, this study's objective was to analyze the physical properties, microbial quality and safety, antioxidant and antimicrobial properties of SMP towards food-borne pathogens.

2. Materials and methods

2.1 Materials

The maturity of mangosteen was determined by the colour (Ketsa and Paull, 2011). The seven stages of maturity for mangosteen are based on the colour of the pericarp: stage 0 (yellowish-white), stage 1 which is the minimum stage for harvesting to export (light greenish-yellow), stage 2 (light greenish-yellow with pink spots), stage 3 (pink), stage 4 (red), stage 5 (red-dish-purple) and stage 6 (dark purple). Mangosteen at stages 5 and 6 are considered to be ripe (Jarimopas *et al*., 2009) and preferable to consumers (Ketsa and Paull, 2011) with mangosteen peel having a water content of 10.31% (Tjahjani *et al*., 2012). For this study, ripe mangosteen (stages 5 and 6) and brown sugar (Gula Prai, Pulau Pinang, Malaysia) were purchased from a local market in Kuala Lumpur and Sri Serdang, Malaysia.

2.2 Fruit sample preparation

The pulps were manually separated from the peels. Then, the peels were washed with running tap water to remove the dirt. Finally, the peels were ground using an electrical blender (Waring Commercial, USA).

2.3 Fermentation of mangosteen peel

In a Schott bottle, brown sugar (100 g) was dissolved in sterile distilled water (1000 mL). Then, the mangosteen peel (100 g) was mixed thoroughly in the mixture. The bottles were closed and left in a dark room for 4 weeks at room temperature (26-29°C) to ferment without agitation. Fermentation was carried out in triplicates.

2.4 Physical properties of spontaneously fermented mangosteen peels

Samples of spontaneously fermented mangosteen peels (SMP) were taken every week from each bottle starting from Week 0 (the starting day of fermentation) and stored in a 4°C chiller. Samples from Week 0 and Week 4 were collected, freeze-dried and stored in a freezer before being analyzed.

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2.4.1 pH measurement

The pH of SMP was tested weekly using a digital pH meter (FE20, Mettler-Toledo AG, Canton of Zurich, Switzerland).

2.4.2 Temperature

The temperature of SMP was measured weekly using a thermometer (Alla LTH-50000P110, France).

2.4.3 Total soluble solid

The total soluble solid of SMP was measured weekly using a refractometer (Atago N1, Atago Co., Ltd, Tokyo, Japan). The readings were recorded as °Brix scores.

2.5 Antioxidant assays

2.5.1 Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu's procedure from Zhang *et al.* (2006) with gallic acid as standard in a 96-well microplate. A total of 20 µL of SMP at Week 0 and Week 4 were diluted and mixed well with 100 µL of Folin:Ciocalteu reagent and left for 5 mins. Then, 70% sodium carbonate solution (80 µL) was added and mixed again. Gallic acid (100 µg/mL) was also prepared as a standard. The plates were covered and left for 2 hrs at room temperature. The absorbance was measured at 750 nm wavelength with a spectrophotometric microplate reader. Each standard and sample were measured as triplicates. A standard curve was plotted using gallic acid and the total phenolic acid was expressed as mg Gallic Acid Equivalents (GAE) per g of sample.

2.5.2 DPPH scavenging assay

DPPH scavenging assay was conducted using a 96-well microplate following the method by Sembiring *et al.* (2018) with a slight modification. In row 1, 100 µL of SMP at Week 0 and Week 4 (10 mg/mL) were added into each well. Dilution was carried out gradually from left to right from row 1 to the last row on the plate. Then, 100 µL of DPPH reagent (0.2 mM) was added to the well and thoroughly mixed. Methanol was used as control and Trolox was used as standard. The plate was wrapped with aluminium foil and left at room temperature for 30 mins. Absorbance was read at 515 nm using a spectrophotometric microplate reader. Each sample and standard were run as triplicates. The percentage scavenging activity was calculated using the formula below:

$$\%\ DPPH\ scavenging = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

The concentration of sample needed to inhibit 50% of DPPH radical (IC_{50} value) for each sample was calculated.

2.6 Microbial counts

2.6.1 Lactic acid bacteria

SMP broth was diluted with 0.1% (w/v) sterile peptone water serially. Then, 30 µL of 10^{-2}, 10^{-4} and 10^{-6} dilutions were inoculated onto De Man, Rogosa and Sharpe agar (MRS) and incubated for 24 hrs at 35°C. The colonies were counted using a colony counter and recorded as $\log_{10}$ colony forming unit (CFU)/mL.

2.6.2 Coliform count

SMP broth was diluted with 0.1% (w/v) sterile peptone water serially. Then, 30 µL of 10^{-2}, 10^{-4} and 10^{-6} dilutions were inoculated onto MacConkey agar and incubated for 24 hrs at 37°C. The colonies were counted using a colony counter and recorded as $\log_{10}$ CFU/mL.

2.6.3 Total plate count

SMP broth was diluted with 0.1% (w/v) sterile peptone water serially. Then, 30 µL of 10^{-2}, 10^{-4} and 10^{-6} dilutions were inoculated onto Plate Count agar and incubated for 24 hrs at 37°C. The colonies were counted using a colony counter and recorded as $\log_{10}$ CFU/mL.

2.7 Bacterial strains and culture

A total of six foodborne pathogens were used: *Bacillus cereus* American Type Culture Collection (ATCC) 10875, *Bacillus subtilis* ATCC 11774, *Clostridium perfringens* ATCC 13124, *Listeria monocytogenes* ATCC 19111, ATCC 13076), *Vibrio parahaemolyticus* ATCC 17802 and *Vibrio vulnificus* ATCC 27562. All bacteria cultures used in this research were sub-cultured on nutrient agar or nutrient agar mixed with 2% of sodium chloride (NaCl) for *V. parahaemolyticus* and *V. vulnificus* and incubated at 37°C for 24 hrs.

2.8 Well diffusion assay

Well diffusion assay of SMP was done according to the Clinical and Laboratory Standard Institute (CLSI), (2018). Bacteria species (10^6 – 10^8 CFU/mL) were spread onto Mueller Hinton agar using a sterile cotton swab. Wells with 8 mm diameter were made using a sterile cork borer and loaded with 50 µL of 20 mg/mL of SMP (dissolved in dimethyl sulfoxide (DMSO)), 0.1% of commercial chlorhexidine (CHX) as positive control and 10% of DMSO as the negative control. The plates were incubated at 37°C for 24 hrs. The diameter of the clear zone around the wells was measured in mm.

2.9 Minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that inhibits

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visible growth while minimum bactericidal concentration (MBC) is the minimum concentration of antimicrobial agent that completely kills bacterial growth. Both parameters were determined according to CLSI (2012). In this study, only SMP at Week 0 and Week 4 was analyzed to compare the effect of fermentation on the mangosteen peel. In a 96-well U-shaped microtiter plate, SMP (20 mg/mL) was mixed and two-fold diluted with Mueller Hinton broth containing inoculum (10^6 – 10^8 CFU/mL). Column 12 had the highest concentration of SMP (10 mg/mL) while Column 3 had the lowest (0.02 mg/mL). Column 1 contained only the medium (negative control) and Column 2 contained only the medium and inoculum (positive control). The plates were incubated at 37°C for 24 hrs. Suspensions (10 µL) from each well were sub-cultured on Mueller Hinton agar to determine the value of MBC. The plates were incubated at 37°C for 24 hrs.

2.10 Time-kill curve assay

A time-kill curve assay was conducted using the previous MIC values obtained. Final concentrations of 0×MIC, 0.5×MIC, 1×MIC, 2×MIC and 4×MIC were prepared by mixing SMP with Mueller Hinton broth containing 10^6 – 10^8 CFU/mL bacterial inoculums for each bacterial species. At 0, 1, 2 and 4 hrs of incubation time, 10 µL suspensions were serially diluted in 1% phosphate buffered saline and spread onto Mueller Hinton agar. The plates were incubated at 37°C for 24 hrs. The results were expressed as $\log_{10}$ CFU/mL.

2.11 Statistical analysis

Using Minitab (Version 16) Statistical Software, a one-way analysis of variance (ANOVA) test was used to determine the significant differences between the variables. Differences with $p \leq 0.05$ were considered significant. All data except MIC and MBC were reported as mean ± standard deviation (SD).

3. Results and discussion

3.1 Physical properties

Table 1 shows the pH, temperature and Brix values of SMP during four weeks of fermentation. The pH of SMP decreased significantly with fermentation time. By Week 4, the pH decreased significantly by 13% to Week 0. The pH of a fermentation system changes as a response to metabolic activities (Zhang *et al.*, 2006) due to the secretion of organic acids by the microorganisms (Sembiring *et al.*, 2018). The fermented plant-based substance commonly has pH values below 4.00 ensuring the product's microbiological safety (Yoon *et al.*, 2005). The pH value of SMP at Week 4 (3.75) was similar to fermented beet juice by *Lactobacillus acidophilus* (3.75) (Raimbault and Tewe, 2001) but was higher than fermented mango peel (3.07) (Kiai and Hafidi, 2014). The temperature reduced slightly throughout the fermentation, with significant changes to Week 3 and Week 4. However, Brix values increased with no significant changes from Week 0 (8.92°Bx) to Week 4 (9.24°Bx).

3.2 Antioxidant properties

Table 2 shows the total phenolic content (TP) and DPPH scavenging activity that is expressed as IC_{50} of SMP. The SMP in Week 4 had a significantly lower TP of 45.35% than the SMP in Week 0. This result, therefore indicated that fermentation reduced the phenolic compounds by degradation and hydrolysis reactions. During fermentation, phenolic compounds are broken down and modified into other molecules such as conjugates and glucosides by the microorganisms (Adebo and Medina-Meza, 2020). One of the possibilities is the oxidation and condensation reaction of single polyphenols to insoluble complex tannins. This reaction is catalyzed by oxidase enzymes or non-enzymatic reactions. Also, some polyphenols may have diffused into the water and contributed to the reduction of phenolic content (Suazo *et al.*, 2014). A similar result was observed in fermented cocoa beans where the phenolic content reduced significantly as much as 62.6% as compared with the non-fermented cocoa beans (Suazo *et al.*, 2014).

DPPH scavenging activity was expressed as IC_{50} which shows the concentration of the sample to reduce 50% of initial DPPH activity (Dessalegn, 2019). In the current study, the IC_{50} for SMP at Week 4 was higher by 32.16% (3.98 to 5.26 µg/mL) as compared with SMP at Week 0. Therefore, SMP at Week 4 showed that the DPPH scavenging activity reduced significantly after 4 weeks of fermentation, mostly caused by lower antioxidant activity. This result corresponds with the reduced TPC of SMP in Week 4 (Table 2). Contradicting results were observed in the IC_{50} values of solid-state

Table 1. Physical properties of spontaneously fermented mangosteen peel (SMP) during the fermentation period.

Physical Properties	Week 0	Week 1	Week 2	Week 3	Week 4
pH	4.31±0.02^a	4.05±0.02^b	3.92±0.00^c	3.89±0.02^c	3.75±0.01^d
Temperature (°C)	29.87±0.07^a	29.81±0.02^a	28.37±0.03^a	28.68±0.14^b	28.48±0.11^{bc}
Brix (°Bx)	8.92±0.22^a	9.18±0.20^a	9.37±0.10^a	9.24±0.14^a	9.24±0.14^a

Values with different superscripts within a row are statistically significantly different at $p \leq 0.05$.

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fermented orange, watermelon and banana peels, where IC_{50} reduced as much as 11.28%, 21.92% and 40.63%, respectively after fermented for seven days (Saleh *et al.*, 2017). The contradicting results were probably due to the loss of volatile compounds in SMP such as hexan-1-ol and hexanal, which was proven to occur in the fermentation of mangosteen juice (Palakawong and Delaquis, 2018). Additionally, the 4-week fermentation period of SMP could be too long that the sugar substrate was depleted and caused the micro-organisms to utilize the antioxidant compounds in SMP. Dessalegn (2019) reported that a long fermentation period may decrease the phenolic compounds because the microorganisms utilize it as a substrate to grow.

Table 2. Total phenolic content (TP) and IC_{50} of spontaneously fermented mangosteen peel (SMP).

	Week 0	Week 4
TP (mg GAE/g)	0.086±0.001[a]	0.047±0.013[b]
IC_{50} (µg/mL)	3.980±0.451[a]	5.263±0.783[a]

Values with different superscripts within a row are statistically significantly different at $p \leq 0.05$.

3.3 Microbial analysis

At Week 0, no LAB and food-borne pathogens were detected (Figure 1). Only total plate count (TPC) was detected at 6.07±1.02 $\log_{10}$ CFU/mL (Figure 1) at Week 0 but increased by 41% at Week 1 but gradually decreased until Week 4. The microorganisms detected in TPC were probably natural aerobic bacteria, yeast, moulds, and fungi on the mangosteen peels. LAB increased significantly from Week 0 (0.00 $\log_{10}$ CFU/mL) to Week 1 (8.43 $\log_{10}$ CFU/mL), indicating that fermentation has taken place, which was proven by the decreased pH between Week 0 and Week 1 (Table 1). However, foodborne pathogens also increased significantly for *E. coli* and *K. pneumoniae* from 7.72 $\log_{10}$ CFU/mL and 7.75 $\log_{10}$ CFU/mL, respectively.

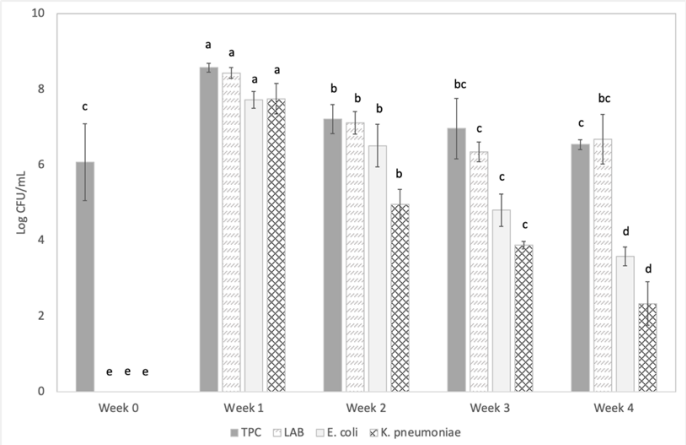

Figure 1. Total plate count (TPC), lactic acid bacteria (LAB), *E. coli* and *K. pneumoniae* of SMP during fermentation. Bars with different notations are statistically significantly different at $p \leq 0.05$.

Starting from Week 2, colonies of food-borne pathogens significantly decreased until they reached 3.58 $\log_{10}$ CFU/mL (*E. coli*) and 2.33 $\log_{10}$ CFU/mL (*K. pneumoniae*) in Week 4. As the fermentation period increased, the food-borne colonies reduced because of the increased acidic environment as indicated in the pH result (Table 1). The system's acidic condition was unfavourable for food-borne pathogens to grow (Yoon *et al*., 2005). Although LAB also decreased significantly, the amount was still higher than the pathogens. Freire *et al.* (2014) showed a similar trend where LAB values of fermented yakupa decreased to approximately one-half log cycle after 30 days of fermentation.

3.4 Well diffusion assay of spontaneously fermented mangosteen peel

Table 3 shows the inhibition zone of SMP at Week 0 and Week 4. All the samples showed a significantly lower inhibition zone than the positive control of the assay (CHX 0.1%). SMP at Week 0 showed inhibition zones ranging from 11.0 to 12.67 mm, with the highest inhibition zones observed in *C. perfringens* (12.67±0.29 mm) and *L. monocytogenes* (12.50±0.00 mm). By Week 4, the effectiveness of SMP on the inhibition zone deteriorated for all bacteria strains. The deterioration was significantly prominent in the inhibition zones of *C. perfringens* (11.8% reduction) and *V. parahaemolyticus* (5% reduction). This result indicated that SMP's antimicrobial property was weaker after fermenting for 4 weeks, probably caused by the breakage of active compounds in the peels during fermentation. The IC_{50} and TPC values for SMP at Week 4 (Table 1) concluded that antioxidants reduced significantly, which further supported the lower inhibition zone of SMP at Week 4. Similarly, solid-state fermentation of *Magnolia afficinalis* for 5 days also showed a lower inhibition zone against *B. cereus* (from 15.5 mm to 15.0 mm) and *L. monocytogenes* (from 16.0 mm to 13.0 mm) (Wen *et al.*, 2013). However, Ramli *et al.* (2017) obtained a lower inhibition zone of *V. parahaemolyticus* (6.67 mm) using *Syzygium polyanthum* leaf extract, probably due to the lower extract concentration used in their study (two times lower). Additionally, active compounds in *S. polyanthum* leaf extract and SMP may differ.

3.5 Minimum inhibitory concentration and minimum bactericidal concentration of spontaneously fermented mangosteen peel

Table 4 shows the MIC and MBC of SMP at Week 0 and Week 4 for selected bacteria strains. The MIC represents the minimum concentration of SMP needed to inhibit visible bacterial growth whereas MBC represents the SMP concentration needed to kill the pathogens. MBC values are commonly higher than MIC values

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Table 3. Well diffusion assay of spontaneously fermented mangosteen peel (SMP) against foodborne pathogens.

Bacteria Strains	Inhibition zone (mm)			
	Week 0		Week 4	
	Positive control (CHX) (0.1%)	Sample (2%)	Positive control (CHX) (0.1%)	Sample (2%)
Bacillus cereus	20	11.17 ± 0.76^{Ab}	20	10.67 ± 0.29^{Ab}
Bacillus subtilis	17.5	11.33 ± 0.29^{Ab}	19	11.00 ± 0.50^{Aab}
Clostridium perfringens	21	12.67 ± 0.29^{Aa}	20.5	11.17 ± 0.29^{Bab}
Listeria monocytogenes	22.5	12.5 ± 0.00^{Aa}	23.5	12.00 ± 0.50^{Aa}
Vibrio parahaemolyticus	21	11.33 ± 0.29^{Ab}	22.5	10.67 ± 0.29^{Bb}
Vibrio vulnificus	19	11.00 ± 0.00^{Ab}	20	10.67 ± 0.29^{Ab}

Values are presented as mean±SD. Values with different uppercase superscripts within a row are statistically significantly different at $p\leq0.05$. Values with different lowercase superscripts within a column are statistically significantly different at $p\leq0.05$. CHX: chlorhexidine.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of spontaneously fermented mangosteen peel (SMP) against foodborne pathogens.

Bacteria Strains	Week 0		Week 4	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Bacillus cereus	0.31	2.50	1.25	10.00
Bacillus subtilis	0.16	0.63	0.31	2.50
Clostridium perfringens	0.31	2.50	0.63	5.00
Listeria monocytogenes	0.16	0.31	0.63	1.25
Vibrio parahaemolyticus	0.31	2.50	2.50	10.00
Vibrio vulnificus	0.16	2.50	0.31	10.00

MBC: minimum bactericidal concentration, MIC: minimum inhibitory concentration.

because higher concentrations of the sample are needed to kill the pathogens.

At Week 0, *B. subtilis*, *L. monocytogenes* and *V. vulnificus* had the lowest MIC (0.16 mg/mL), whereas *L. monocytogenes* and *B. subtilis* had the lowest MBC of 0.31 mg/mL and 0.63 mg/mL, respectively. In Week 4, both MIC and MBC for all strains increased by two to four times as compared with Week 0. MIC at Week 4 for *B. subtilis* and *V. vulnificus* was the lowest (0.31 mg/mL), followed by *C. perfringens* and *L. monocytogenes* with 0.63 mg/mL each. The *L. monocytogenes* had the lowest MBC value at Week 4 with only 1.25 mg/mL.

For both weeks, both MIC and MBC values increased after fermentation, indicating higher SMP concentration was needed to inhibit and kill the pathogens. SMP was the most effective in inhibiting *B. subtilis*, *V. vulnificus* and especially, *L. monocytogenes*. The latter pathogen was inhibited and killed by using the lowest concentration of SMP at both Weeks 0 and 4. However, the concentration of SMP at Week 4 used in this study was still higher than *S. polyanthum* leaf extract which only needed 0.63 mg/mL to kill *L. monocytogenes* (Ramli *et al*., 2017). Hence, SMP at Week 0 showed better performance, probably due to intact active compounds that may be more effective than SMP at Week 4 where the active compounds had been broken down, hence lowering the antibacterial effects. This result was positively correlated with the TPC (Table 2) and well diffusion assay (Table 3) results.

3.6 Killing time curve assay

The ability of SMP at Week 0 and Week 4 to kill food-borne pathogens was studied using a killing time assay (Figure 2). The concentration of SMP at Week 0 and Week 4 was based on 0, 0.5, 1, 2 and 4 times the MIC values for each bacterium obtained from Table 3. Among all the pathogens, SMP at Week 0 and Week 4 killed only *L. monocytogenes* and *V. parahaemolyticus* at 4 times of each respective MIC (4×MIC). SMP at Week 0 with 4×MIC (0.64 mg/mL) killed *L. monocytogenes* within 2 hrs (Figure 2a), whereas SMP at Week 4 with 4×MIC (2.52 mg/mL) had a killing time of 1 hr (Figure 2b). For *V. parahaemolyticus*, SMP at Week 0 with 4×MIC (1.24 mg/mL) had a killing time of 2 hrs (Figure 2c). SMP at Week 4 with 2×MIC concentration (5.00 mg/mL) killed *V. parahaemolyticus* within 4 hrs, whereas at a higher concentration of 4×MIC (10.00 mg/mL) shortened the killing time to only 2 hrs (Figure 2d). Due to the higher concentration of SMP extract exposed to the bacteria cell, the membrane becomes leaky to cytoplasmic components and the cell wall degenerates, causing the cell to die (Gangoue-Pieboji *et al.*, 2009; Bubonja-Sonje *et al.*, 2011). Sample extract needs to

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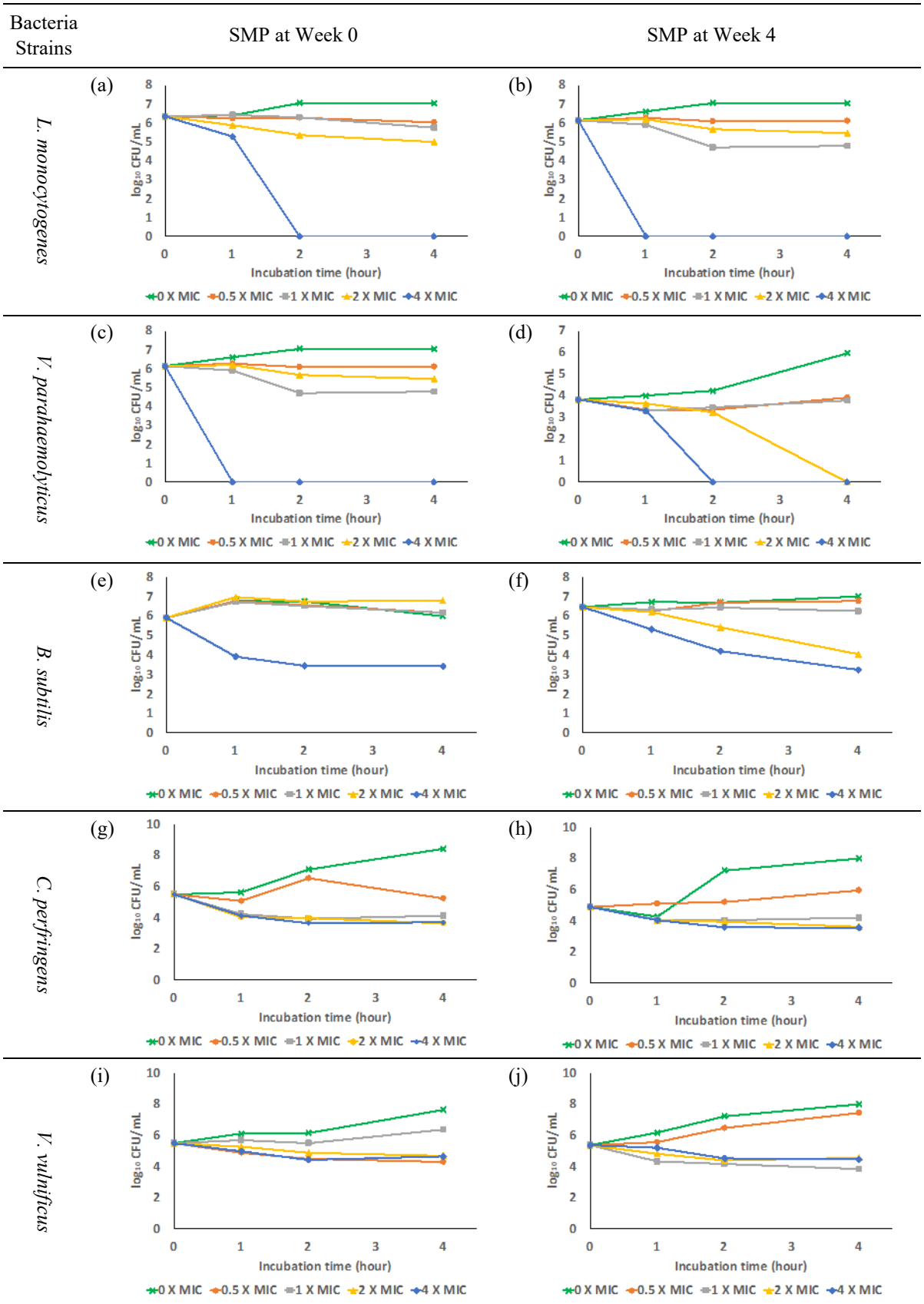

Figure 2. (a) Time-kill curve plots for *L. monocytogenes* (0, 0.08, 0.16, 0.32 and 0.64 mg/mL) after exposed to SMP at Week 0. (b) Time-kill curve plots for *L. monocytogenes* (0, 0.32, 0.63, 1.26 and 2.52 mg/mL) after exposed to SMP at Week 4. (c) Time-kill curve plots for *V. parahaemolyticus* (0, 0.16, 0.31, 0.62 and 1.24 mg/mL) after exposed to SMP at Week 0. (d) Time-kill curve plots for *V. parahaemolyticus* (0, 1.25, 2.50, 5.00 and 10.00 mg/mL) after exposed to SMP at Week 4. (e) Time-kill curve plots for *B. subtilis* (0, 0.08, 0.16, 0.32 and 0.64 mg/mL) after exposed to SMP at Week 0. (f) Time-kill curve plots for *B. subtilis* (0, 0.16, 0.31, 0.62 and 1.24 mg/mL) after exposed to SMP at Week 4. (g) Time-kill curve plots for *C. perfringens* (0, 0.08, 0.16, 0.32 and 0.64 mg/mL) after exposed to SMP at Week 0. (h) Time-kill curve plots for *C. perfringens* (0, 0.08, 0.16, 0.32 and 0.64 mg/mL) after exposed to SMP at Week 4. (i) Time-kill curve plots for *B. cereus* (0, 0.16, 0.31, 0.62 and 1.24 mg/mL) after exposed to SMP at Week 0. (j) Time-kill curve plots for *B. cereus* (0, 0.63, 1.25, 2.50 and 5.00 mg/mL) after exposed to SMP at Week 4. Values given in the brackets after species are 0×MIC, 0.5×MIC, 1×MIC, 2×MIC and 4×MIC, respectively.

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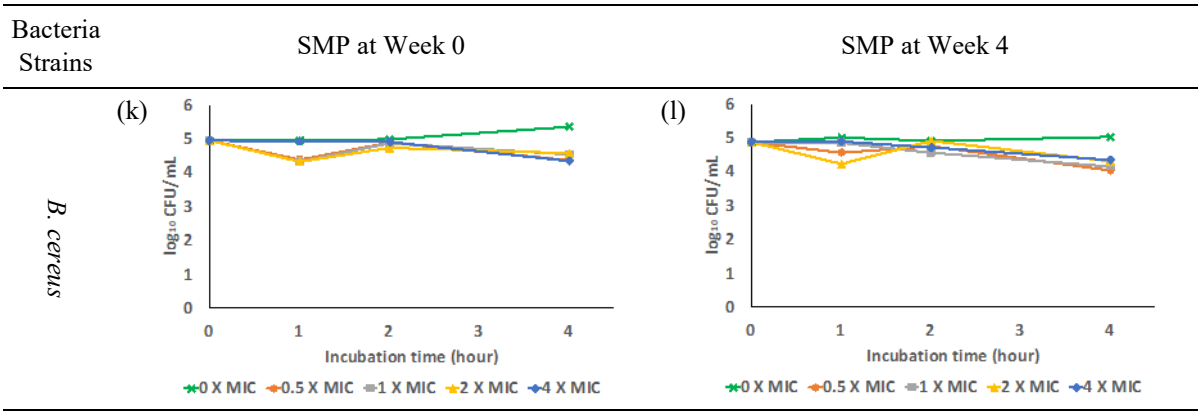

Figure 2 (Cont.). (k) Time-kill curve plots for *V. vulnificus* (0, 0.16, 0.31, 0.62 and 1.24 mg/mL) after exposed to SMP at Week 0. (l) Time-kill curve plots for *V. vulnificus* (0, 0.16, 0.31, 0.62 and 1.24 mg/mL) after exposed to SMP at Week 4. Values given in the brackets after species are 0×MIC, 0.5×MIC, 1×MIC, 2×MIC and 4×MIC, respectively.

bind, occupy, and remain at the target site for sufficient time to interfere with the bacteria's chemical reactions that prevent the metabolic process and kill the bacteria (Gangoue-Pieboji *et al.*, 2009).

SMP at Week 0 and Week 4 significantly reduced the *B. subtilis* (Figures 2e and 2f) and *C. perfringens* (Figures 2g and 2h) colonies with increasing incubation time. For *B. subtilis*, SMP at Week 0 with 4 times MIC concentration (0.64 mg/mL) managed to decrease the number of colonies by 50%, whereas 2×MIC (0.32 mg/mL) and 4×MIC (0.64 mg/mL) concentration of SMP at Week 4 reduced the colonies by 39% and 54%, respectively. SMP at both Weeks 0 (Figure 2g) and Week 4 (Figure 2h) with 1×MIC, 2×MIC and 4×MIC significantly reduced the *C. perfringens* colonies to approximately 3 $\log_{10}$ CFU/mL (33% reduction) by 2 hrs, which remained stagnant until the end of incubation time. SMP at Week 0 was only effective in reducing 33% of the *V. vulnificus* colonies with 0.5×MIC, 2×MIC and 4×MIC (Figure 2i), whereas SMP at Week 4 showed a better performance with 1×MIC, 2×MIC and 4×MIC (Figure 2j). In contrast, SMP at Week 0 and Week 4 was less effective in reducing the colonies of *B. cereus* (Figures 2k and 2l). Regardless of the MIC concentration, SMP at both weeks reduced the *B. cereus* colonies by only 20% (Figures 2i and 2j).

4. Conclusion

In conclusion, SMP has antibacterial activity against a wide spectrum of food-borne pathogens specifically *B. cereus*, *B. subtilis*, *C. perfringens*, *L. monocytogenes*, *V. parahaemolyticus* and *V. vulnificus*. However, SMP was only lethal to *L. monocytogenes* and *V. parahaemolyticus*, depending on the concentration used. The results showed that SMP at Week 0 had better antibacterial activity than SMP at Week 4, indicating that the active compounds in the unfermented mangosteen peel were more effective in inhibiting and killing pathogenic bacteria. The 4 weeks fermentation period may have caused a reduction of antioxidants in the SMP as shown in the TP and DPPH scavenging assay. Therefore, it is recommended that the spontaneous fermentation of mangosteen peel needs further study at a shorter interval to identify the optimum fermentation time for the highest production of antioxidant compounds and antimicrobial effects.

Conflict of interest

The authors declare no conflict of interest.

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References

Adebo, O.A. and Medina-Meza, I.G. (2020). Impact of Fermentation on the Phenolic Compounds and Antioxidant Activity of Whole Cereal Grains: A Mini Review. *Molecules*, 25(4), 927. https://doi.org/10.3390/molecules25040927

Agamuthu, P. (2009). Challenges and opportunities in agro-waste management: An Asian perspective, presented at Inaugural Meeting of First Regional 3R Forum in Asia 11-12 November. Tokyo, Japan.

Arekemase, M.O., Adetitun, D.O. and Ahmed, M.I. (2020). Biochemical analysis of some fruit peels and comparison of Lactic acid production by authochthonous Lactic acid bacteria using fruit peels. *Sri Lankan Journal of Biology*, 5(1), 15-26. http://doi.org/10.4038/sljb.v5i1.53

Bubonja-Sonje, M., Giacometri, J. and Abram, M. (2011). Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. *Food Chemistry*, 127(4), 1821-1827. https://doi.org/10.1016/j.foodchem.2011.02.071

Cantatero, V., Filannino, P., Gambacorta, G., Pasquale, I.D., Pan, S., Gobbeti, M. and Cagno, R.D. (2019). Lactic acid fermentation to re-cycle apple by-products for wheat bread fortification. *Frontiers in Microbiology*, 10, 2574. https://doi.org/10.3389/fmicb.2019.02574

Chanprasartsuk, O., Prakitchaiwattana, C., Sanguandeekul, R. and Fleet, G.H. (2010). Autochthonous yeasts associated with mature pineapple fruits, freshly crushed juice and their ferments; and the chemical changes during natural fermentation. *Bioresource Technology*, 101(19), 7500-7509. https://doi.org/10.1016/j.biortech.2010.04.047

Chelule, P.K., Mokoena, M.P. and Gqaleni, N. (2010). Advantages of traditional lactic acid bacteria fermentation of food in Africa. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 2(2), 1160-1167

Clinical and Laboratory Standards Institute (CLSI). (2018). Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. (CLSI Standard M07). Wayne, Pennsylvania, USA: CLSI.

Cunha, B.L., Franca, J.P., Moraes, A.A., Chaves, A.L., Gaiba, S., Fontana, R., Sacremento, C.K., Ferreira, L.M. and Franca, J.P. (2014). Evaluation of antimicrobial and antitumoral activity of *Garcinia mangostana L.* (mangosteen) grown in Southeast Brazil. *Acta Cirurgica Brasileira*, 29(2), 21-28. https://doi.org/10.1590/s0102-86502014001400005

Dessalegn, E. (2019). Effect of traditional fermentation time on total phenolic content and in vitro antioxidant activity of kocho: Ethiopian fermented food from Enset (*Enssete ventricusum welw. Chessman*). *Food Science and Quality Management*, 91, 44-51. https://doi.org/10.7176/FSQM/91-05

Freire, A.L., Ramos, C.L., Almeida, E.G., Duarte, W.F. and Schwan, R.F. (2014). Study of the physicochemical parameters and spontaneous fermentation during the traditional production of yakupa, an indigenous beverage produced by Brazilian Amerindians. *World Journal of Microbiology and Biotechnology*, 30(2), 567-577. https://doi.org/10.1007/s11274-013-1476-0

Gangoue-Pieboji, J., Eze, N., Djintchui, A.N., Ngameni B., Tsabang, N., Pegnyemb, D.E., Biyiti, L., Ngassam, P. Koulla-Shiro, S. and Galleni, M. (2009). The in-vitro antimicrobial activity of some traditionally used medicinal plants against beta-lactam-resistant bacteria. *The Journal of Infection in Developing Countries*, 3(9), 671–680. https://doi.org/10.3855/jidc.77

Goh, C.H. (2018). Green energy source going to waste. The Star. Retrieved from The Star website: https://www.thestar.com.my/business/smebiz/2018/09/24/green-energy-source-going-to-waste

Gutiérrez, S., Martinez-Blanco, H., Rodriguez-Aparicio, L.B. and Ferrero, M.A. (2016). Effect of fermented broth from lactic acid bacteria on pathogenic bacteria proliferation. *Journal of Dairy Science*, 99(4), 2654-2665. http://dx.doi.org/10.3168/jds.2015-10439

Hien, N.T. Hoa, H.D., Hoa, N.Q., Tram, P.T. and Tu, N.T.M. (2018). Study on extraction of tannins from the *Garcinia mangostana* Linn peel in Vietnam. *Vietnam Journal of Science and Technology*, 56(4A), 113-120. https://doi.org/10.15625/2525-2518/56/4a/13118

Hur, S.J., Lee, S.Y., Kim, Y.C., Choi, I. and Kim, G.B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*, 160, 346-356. https://doi.org/10.1016/j.foodchem.2014.03.112

Iralapati, V. and Kummari, S. (2014). Production of citric acid from different fruit peels using *Aspergillus niger*. *International Journal of Scientific Engineering and Research*, 3(5), 129-130.

Jarimopas, B., Pushpariksha, P. and Sing, S.P. (2009). Postharvest damage of mangosteen and quality grading using mechanical and optical properties as indicators. *International Journal of Food Properties*, 12(2), 414-426. https://doi.org/10.1080/10942910701837262

Jindarat, S. (2014). Xanthones from mangosteen (*Garcinia mangostana*): multi-targeting pharmacological properties. *Journal Medical Association of Thailand*, 97(2), SI96-S20I.

Kannahi, M. and Elangeswari, S. (2015). Enhanced production of cellulose on different fruit peel under submerged fermentation. *International Journal of Pharmaceutical Sciences Review and Research*, 32 (2), 161-165.

Kaomongkolgit, R., Jamdee, K. and Chaisomboon, N. (2009). Antifungal activity of alpha-mangostin against *Candida albicans*. *Journal of Oral Science*, 1(3), 401-406. https://doi.org/10.2334/josnusd.51.401

Ketsa, S. and Paull, R.E. (2011). Mangosteen (*Garcinia mangostana L.*). In Yahia, E.M. (Ed.) Postharvest Biology and Technology of Tropical and Subtropical Fruits, p. 1-32. Cambridge, United Kingdom: Woodhead Publishing. https://doi.org/10.1533/9780857092618.1

Kiai, H. and Hafidi, A. (2014). Chemical composition changes in four green olive cultivars during spontaneous fermentation. *LWT – Food Science and Technology*, 57(2), 663-670. https://doi.org/10.1016/j.lwt.2014.02.011

Liu, S., Han, L. and Zhou, Z. (2011). Lactic acid bacteria in traditional fermented Chinese foods. *Food Research International*, 44(3), 64-61. https://doi.org/10.1016/j.foodres.2010.12.034

Palakawong, C. and Delaquis, P. (2018). Mangosteen processing: A review. *Journal of Food Processing and Preservation*, 42, e13744. https://doi.org/10.1111/jfpp.13744

Raganati, F., Procentese, A., Olivieri, G., Russo, M.E. and Marzocchella, A. (2016). Butanol production by fermentation of fruit residues. *Chemical Engineering Transactions*, 49, 229-234.

Raimbault, O.A. and Tewe, O.O. (2001). Protein enrichment of sweet potato by solid substrate fermentation using four monoculture fungi. *Nigerian Journal of Biotechnology*, 9(1), 1–4.

Ramli, S., Son, R., Shaari, K. and Rukayadi, Y. (2017). Antibacterial activity of ethanolic extract of *Syzygium polyanthum L.* (salam) leaves against foodborne pathogens and application as food sanitizer. *BioMed Research International*, 2017, 9024246. https://doi.org/10.1155/2017/9024246

Reddy, L.V., Reddy, O.V. and Wee, Y. (2011). Production of ethanol from mango (*Mangifera indica L.*) peel by *Saccharomyces cerevisiae* CFTRI101. *African Journal of Biotechnology*, 10 (20), 4183-4189

Rohman, A., Rafi, M., Alam, G., Muchtaridi, M. and Windarsih, A. (2019). Chemical composition and antioxidant studies of underutilized part of mangosteen (*Garcinia mangostana L.*) fruit. *Journal of Applied Pharmaceutical Science*, 9(8), 47-52. https://doi.org/10.7324/JAPS.2019.90807

Sadef, Y., Javed, T., Javed, R., Mahmood, A., Alwahib,i M.S., Elshikh, M.S., Abdel Gawwa, M.G., Alhaji, J. and Rasheed, R.A. (2022) Nutritional status, antioxidant activity and total phenolic content of different fruits and vegetables' peels. *PLoS ONE*, 17 (5), e0265566. https://doi.org/10.1371/journal.pone.0265566

Saleh, R.M., Kabli, S.A., Al-Gami, S.M., Mohamed, S.A. and Almulaiky, Y.Q. (2017). Enhancement of the total phenolic and flavonoid contents and antioxidant activity of some fruit peels by solid state fermentation of *Trichoerma* V6. *Journal of King Abdulaziz University*, 29(2), 13-20. https://doi.org/0.4197/Sci.29-2.3

Sembiring, E.N., Elya, B. and Sauriasari, R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) roxb. *Pharmacognosy Journal*, 10(1), 123-127. https://doi.org/0.5530/pj.2018.1.22

Singh, A.K., Rath, S., Kumar, Y., Masih, H., Peter, J.K., Benjamin, J.C., Singh, P.K. and Singh, P. (2014). Bio-ethanol production from banana peel by simultaneous saccharification and fermentation process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae. International Journal of Current Microbiology and Applied Sciences*, 3(5), 84 -96

Suazo, Y., Davidov-Pardo, G. and Arozarena, I. (2014). Effect of fermentation and roasting on the phenolic concentration and antioxidant activity of cocoa from Nicaragua. *Journal of Food Quality*, 37(1), 50-56. https://doi.org/10.1111/jfq.12070

Tjahjani, S., Widowati, W., Khiong, K., Suhendra, A. and Tjokropranoto, R. (2012). Antioxidant properties of *Garcinia mangostana L* (mangosteen) rind. *Procedia Chemistry*, 13, 198-203. https://doi.org/10.1016/j.proche.2014.12.027

Wen, Y.-L., Yan, L.-P. and Chen, C.-S. (2013). Effects of fermentation treatment in antioxidant and antimicrobial activities of four common Chinese herbal medicinal residues by *Aspergillus oryzae*. *Journal of Food and Drug Analysis*, 21(2), 219-226. https://doi.org/10.1016/j.jfda.2013.05.013

Wonghirundecha, S. and Sumpavapol, P. (2012). Antibacterial activity of selected plant by-product against food-borne pathogenic bacteria. In 2012 International Conference on Nutrition and Food Sciences IPCBEE. Vol. 39, p. 116-120. Singapore: IACSIT Press.

Yoon, K.Y., Woodams, E.E. and Hang, Y.D. (2005). Fermentation of beet juice by beneficial lactic acid bacteria. *LWT-Food Science and Technology*, 38(1), 73-75. https://doi.org/10.1016/j.lwt.2004.04.008

Zhang, Q. Zhang, J., Shen, J., Silva, A. and Dennis, D.A. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweed. *Journal of Applied Phycology*, 18, 445-450. https://doi.org/10.1007/s10811-006-9048-4

