Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(SI-2): 153-165. **OPEN ACCESS**

Antioxidant and antimicrobial properties of yogurt incorporated with Mango (*Mangifera indica* L.) leaves and their effects on the viability of Lactic acid bacteria (LAB) during storage

Nurul Husna Sarfuddin[1], Mohd Nizam Lani[1,2], Fauziah Tufail Ahmad[1,2*]

[1]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, **Malaysia**
[2]Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, **Malaysia**

***Correspondence: fauziah.tufail@umt.edu.my** **Received 05-07-2021, Revised: 12-08-2021, Accepted: 15-08-2021 e-Published: 19-08-2021**

Mango (*Mangifera indica*) is an alternative herbal medicine that has been used for decades. Different parts of mango tree have been applied in enthno medicinal but rarely used in food products. This study mainly focus on antioxidant and antimicrobial properties of yogurt incorporated with mango leaf extract (MLE) and their effect on the microbial count and viability of lactic acid bacteria. The antioxidant properties of MLE and yogurt incorporated with the extract was carried out by analyzing the total phenolic content (TPC) and 1,1- diphenyl-2-picrylhdrazyl (DPPH). The acidity content of yogurt was determined using pH and titratable acidity (TA). The antimicrobial test of MLE was determined using minimum inhibition concentration (MIC) and microbial count was calculated using total plate count. Result showed that 30% of the mango leaves extract has the highest value in DPPH and TPC compared to 20% and 25% extract. The inhibitory properties against selective pathogens were also exhibited. The incorporation of MLE into yogurt retained the antioxidant properties in the product ($p < 0.05$). The growth of bacteria, yeast and mold were not affected by the incorporation of MLE. There was also no reduction of lactic acid bacteria in all treatments indicating the lactic acid bacteria were viable until the end of storage. The treated yogurt also showed higher acidity ($p < 0.05$) during storage time. In conclusion, the incorporation of mango leaf extract into yogurt significantly increased the antioxidant properties and tend to increase the stability of lactic acid bacteria to the recommended concentration (6 $\log_{10}$ CFU/g) in the product.

Keywords: Mango leaves, yogurt, antioxidant, antimicrobial, viability, lactic acid bacteria

INTRODUCTION

Mango leaves are high in antioxidant properties which mangiferin is the main phenolic compound (Fernandez et al. 2013). Mangiferin is a polyphenolic antioxidant which reported with excellent antioxidant power, reduce lipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities (Shah et al. 2010). Leaves, barks, and fruit peel of mango are rich in mangiferin than the edible of mango pulp (Luo et al. 2012). Mango leaves were reported to have a potent antimicrobial agent that contain glucoside and possess antiviral activity against herpes simplex type 2 viruses, hypoglycemic, anti-hyperlipidemic and antiatherogenic activities (Hannan et al. 2013). Mango leaves were also reported with antimicrobial activities against

Streptococcus pneumoniae, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Candida albicans* and other *Enterobacteriaceae* (Olasehinde et al. 2018; Gied et al. 2012).

Incorporation of antimicrobial and antioxidant properties from the natural source such as mango leaf extract can assist to increase the quality and shelf life of food products but may also inhibit the beneficial microbes in fermented products such as yogurt. Therefore, studies need to be done to study the mechanism of addition plant extract towards the viability of beneficial microbes such as lactic acid bacteria. There were some studies reported on the effect of variety of plant extract on the quality of fermented products. Shafiee et al. (2010) reported on the antioxidant from the plant extract tends to increase the shelf life of starter culture in yogurt due to the declining of beneficial microbe in yogurt during storage which is caused by buffering capacity. Shori (2013) found the incorporation of soybean into yogurts improve the viability of lactic acid bacteria and antioxidant activity. Yogurts supplemented with mixture of plant extracts such as olive, garlic, onion and citrus also showed greater buffering ability and maintained greater lactic acid bacteria counts (Michael et al. 2015). These studies indicated that the addition of plant extract not only can increase the antioxidant properties of food, but also influence the growth and activity of lactic acid bacteria and subsequently increase the functional properties of fermented food product.

MATERIALS AND METHODS

Samples preparation of mango leaf

Matured mango leaves were washed and cut into small pieces. Then, samples were dried at 45℃ and ground into powder. A total of 100 g of mango leaf powder was boiled with 1500 mL distilled water for 2 hours. Samples were then filtered using filter paper (Whatman No. 4). Then, the filtrate was concentrated at 60℃. After that, the extracts were stored at 18℃ for further analysis (Seo et al. 2014). Plant aqueous extracts from different concentrations (20%, 25%, 30%) that contains the highest antioxidant properties was selected to be added into the yogurt.

Preparation of yogurt with mango leaf extract

Fresh milk was heated to a temperature of 85℃ double using boiler method and stirred homogeneously. Sample was cooled with cold water bath until the temperature dropped to 43℃. Then, 3% (w/v) starter culture of commercial plain yogurt and 5% (w/v) of mango leaf extract with selected concentration were added into the milk and stirred homogeneously. Yogurt without the addition of mango leaf extract was used as control. The mixtures were incubated in the incubator at 37℃ before the yogurts were cooled at 4℃ prior to further analysis of antioxidant, chemical and antimicrobial. These analyses were done on the samples at 4 day intervals for 16 days of storage (Shima et al. 2012).

Isolation of lactic acid bacteria (LAB)

A total of 10 g of commercial yogurt were homogenised in 90 mL of Buffered Peptone Water (Oxoid, UK). Then, serial dilution was performed. After that, 0.1 mL of 10^{-3} to 10^{-5} yogurt dilutions were transferred to MRS agar (Oxoid, UK) supplemented with 0.8% $CaCO_3$ and M17 Agar (Oxoid, UK) and incubated under anaerobic conditions at 30°C for 48 hours. Then, the colonies were enumerated and CFU/g of LAB count were determined. LAB were validated based on their microscopic and biochemical features by performing Gram staining, catalase test and API 50 CHL Kit (Salleh et al. 2021; Qian et al. 2018).

Gram staining test

Gram staining was done as described by Harvey et al. (2007) and examined under a light microscope (Leica DME, Matrix Optics (M) Sdn. Bhd.) using 1000X magnification (oil immersion).

Catalase test

The growth the colonies must be from 18-24 hours culture and cultures used should be exposed to air prior to testing (Perilla, 2003). Hydrogen peroxide (3-4 drops) were added on the bacteria to observe whether the bubble formation were present or not. Immediate bubble formation represents a positive reaction to the catalase test. Since lactic acid bacteria are catalase-negative organisms, thus the result must not have any vigorous bubbling (Sherman, 2011).

Identification of lactic acid bacteria (LAB) using API 50 CHL Kit

The colonies of LAB on MRS and M17 agar were inoculated into 2 mL of sterile saline water containing 0.85% (w/v) NaCl and compared with

3 McFarland for standard turbidity. The colonies of unknown were identified with API 50 CHL kit (bioMerieux, France). The colour changes for 50-wells carbohydrate fermentation strips of the unknown isolates were determined after incubation at 35°C for 48 hours. Then, API profiles were analysed using API profile index number. The unknown LAB were identified with the percentage of probability of species identification using APIWEB ™ (Salleh et al. 2021).

Free Radical Scavenging Activity (DPPH)

The method used for DPPH assay is described by Sharma and Bhat (2009). Distilled water was used as blank which was similar solvent for extracted sample. Control was a mixture of DPPH solution with distilled water. Next, 0.1 mM of DPPH (1,1-diphenyl-2-picryl-hydrazyl) was prepared in mixture of ethanol. A total of 0.6 mL of extract was mixed with 4.5 mL of DPPH solution and kept in the dark for 20 minutes. Absorbance of the mixture was analysed using spectrophotometer at 517 nm. The percentage of inhibition (% of inhibition) was determined using the formula:

$$Percentage\ of\ inhibition(\%) = \frac{Absorbance\ of\ control\ (A_0) - Absorbance\ of\ sample\ (A_c)}{Absorbance\ of\ control\ (A_0)}$$

Total Phenolic Content (TPC)

Total phenolic compounds (TPC) was calculated as gallic acid (GA) equivalents (µg mL^{-1}) (Ismail, et al. 2004). A total of 0.2 mL was mixed with 6ml of distilled water and 0.5 mL of Folin-Ciocalteu's for 5 minutes. Then, 1.5 mL of 20% sodium carbonate was added and diluted with distilled water to a final volume of 10 mL. The mixtures were vortexed and left in the dark room for 2 hours. The absorbance was determined by using spectrophotometer at 760 nm. Gallic acid was used as standard for this TPC test.

Minimum Inhibition Concentration (MIC)

Minimum inhibitory concentrations (MICs) were done to measure the inhibition of the extract at the lowest concentration against LAB and *Staphylococcus aureus* was determined after overnight incubation (Karaman et al. 2003). The inhibition was calculated based on this calculation:

$$Percentage\ inhibition: \frac{optical\ density\ in\ control - optical\ density\ in\ test\ set}{optical\ density\ in\ control} \times 100\%$$

Aerobic Plate Count

A total of 10 mL of yogurt incorporated with 0.05 g of mango leaf extract was used. Then, this sample was added into 90 mL of 0.1% of peptone water and mixed well for serial dilution. Next, 0.1 mL of the sample from each dilution was plated on plate count agar (PCA) by spread plate method. The plates were incubated at 37±1°C for 24±2 hours (Lani et al. 2017). After incubation, the number of colonies on the plates were enumerated based on the following formula:

$$Colony\ forming\ unit\ (CFU/g) = \frac{Number\ of\ colony}{Dilution\ factor \times Volume\ of\ culture\ plate}$$

Mold and Yeast Count

A total of 10 mL of yogurt incorporated with 0.05 g of mango leaf extract was added into 90 mL of 0.1% of peptone water for serial dilution. Next, 0.1 mL of each dilution was plating on acidified potato dextrose agar (PDA) with 10% tartaric acid by spread plate method. The plates were incubated at 25±2°C for 5 days and the colonies were enumerated to determine CFU/g (Lani et al. 2019).

pH value

The pH value of sample was determined using a pH meter (InoLab, WTW Series, Model 720). This instrument was calibrated using buffer solution (WTW, Model STP 7) with pH 4, 7 and 10 before usage. The electrode was rinsed with distilled water and wiped with tissue after each reading.

Titratable Acidity (TA)

A total of 20 mL of yogurt and 1 ml of 2% w/v solution of phenolphthalein was titrated with 0.1 M sodium hydroxide solution until it changed to pink colour. The pink colour was formed an indication that the sample had achieved its titratable acidity level (Tomovska et al. 2016).

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel and Minitab software at confidence level at $\alpha \leq 0.05$. Significant difference at ($p<0.05$) was done with one-way ANOVA and comparison of the differences between each pair of mean was carried out via

Tukey HSD.

RESULTS

Identification of lactic acid bacteria (LAB)

The colony of bacteria was observed for their microscopic, morphological and biochemical characteristics through catalase test and Gram stain (Table 1). For catalase test, there was no bubbling when a few drops of hydrogen peroxide were added which indicates that it was a negative catalase result. This result was supported by Ismail et al. (2018), who stated that LAB cannot change hydrogen peroxide into water and oxygen as it does not produce catalase enzymes and does not require oxygen to grow because it is an anaerobic bacteria. Based on the morphological study using Gram stain, all isolates are Gram-positive rod and cocci, which indicate the isolated bacteria were LAB. Using API 50 CHL kit, the unknown bacteria in the yogurt were identified as *Lactobacillus delbrueckii* ssp*. delbrueckii* (74.8% probability) and *Streptococcus salivarius* ssp. *thermophiles* (94.2% probability). The percentage shown was the probability of species identification based on the database of APIWEB ™. The similar species was also found by Lestari et al. (2018) and Linares et al. (2016) in their yogurt fermentation.

Antioxidant properties of mango leaves extract (MLE)

Free radical scavenging activity (DPPH) and total phenolic content

In general, there was a significant effect ($p < 0.05$) of mango leaf extract on different concentrations of inhibition to prevent oxidation (Figure 1). This shows that the concentration of 30% extract had the highest percentage inhibition indicating that plants generally may have phenolic and flavonoid content which that exhibit the free radical scavenging activity (Kingne et al. 2018). Trend of the result showed the increasing of scavenging activity with the higher concentration was in agreement with Bagchi and Puri (1998) who reported that the higher the plant concentration will produce greater hydrogen –donating ability, thus exhibit the higher antioxidant activity. The findings are parallel with the study by Kingne et al. (2018) and Lin et al. (2008) who found that the matured mango leaves in hot aqueous extract were higher in radical scavenging activity than young leaves in hot aqueous extract. Mango leaf extract has high DPPH value due to phenolic compounds and the water is more polar to enhance its ability to extract polar compounds (Seo et al. 2014). The use of water as a solvent will not only reduce the cost of the process but also make the process eco-friendly (Kulkarni and Rathod 2016).

Similar with DPPH value, the trend of the total phenolic content also showed the significant increasing of the phenolic content ($p<0.05$) with the increasing concentrations of the extracts (20%, 25% and 30%) (Figure 2). This result indicated that phenolic was the potential contributor to the high antioxidant activity in mango leaf extract (Kingne et al. 2018).

Minimum Inhibition Concentration (MIC)

There were inhibitory properties showed by mango leaves extract (MLE) but without significance different ($p>0.05$) among all concentrations. This indicates while killing the pathogen which was *S. aureus,* MLE also slightly affected the viability of the LAB. There was no significant difference ($p>0.05$) between the different plant extract concentrations against *S. aureus*. However, there is a tendency that the higher the concentration of the plant extract showed higher percentage inhibition. Study by Kamlumbi (2018) also found that MLE have the potential to effectively treat any *S. aureus* - related infections. The inhibitory properties of MLE potentially contributed by phytoconstituents such as flavonoids, tannins, coumarins, alkaloids, terpenoids and phenolics which normally inhibit growth of Gram-positive bacteria (Kamlumbi (2018).

Storage Stability Study on Yogurt

Storage stability study was carried out on yogurt controls (without mango leaves extract) and yogurt with 30% of mango leaves extract (MLE) for 16 days at 4℃. The yogurt samples were taken over 4 days interval and analysed with DPPH assay, total phenolic content, pH determination, titratable acidity and microbiological analysis.

Table 1: Identification of lactic acid bacteria

Strains	Catalase test	Gram staining	API® 50 CHL	
			Identified organism	**Probability**
MRS agar	Negative	Gram-positive rod	*Lactobacillus delbrueckii* ssp. *delbrueckii*	74.8% Probability
M17 agar	Negative	Gram-positive cocci	*Streptococcus salivarius* ssp. *thermophilus*	94.2% Probability

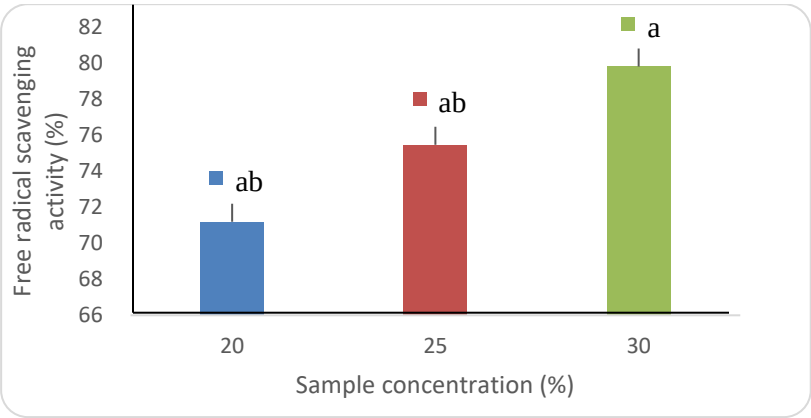

Figure 1: The DPPH value of different concentration of mango leave extract. Different letter indicate significant different (p<0.05) between all sample concentration.

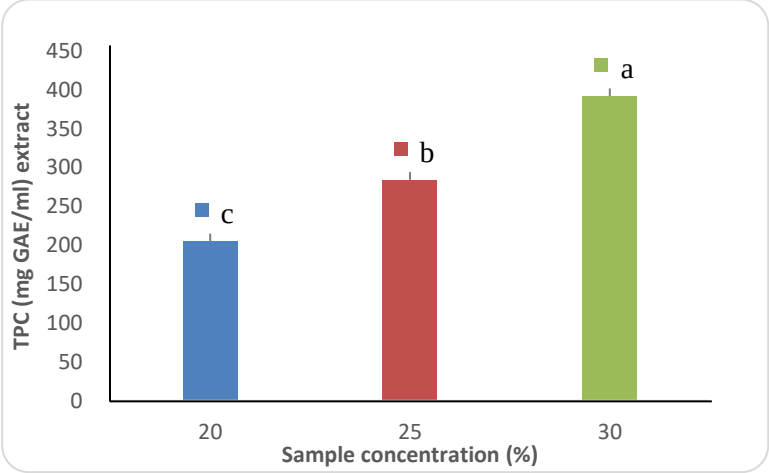

Figure 2: The total phenolic content of different concentration of mango leave extract. Different letter indicate significant different (p<0.05) between all sample concentration

Table 2: Minimum Inhibition Concentration of the mango leaves extract against *L. delbrueckii, S. thermophiles* and *S. aureus*

SAMPLE	MICROORGANISMS (%)		
	Lactobacillus delbrueckii	***Streptococcus thermophilus***	***Staphylococcus aureus***
20% extract	6.84±0.04^{Aa}	5.15±0.05^{Aa}	17.67 ± 7.62^{Aa}
25% extract	3.57±0.03^{Aa}	4.56±0.06^{Aa}	22.23 ± 12.7^{Aa}
30% extract	2.64±0.06^{Aa}	3.77±0.01^{Aa}	34.52 ± 1.34^{Aa}

*Different capital letter represents significant difference between rows. Different small letter represents significant difference between columns

Free Radical Scavenging Activity (DPPH) in yogurt

Mango leaf extract (MLE) significantly affected ($p<0.05$) the antioxidant activity in yogurt during storage (Figure 3). The antioxidant activity in yogurt without MLE was not stable during storage ($p<0.05$). In the contrary, yogurt with MLE constitute a feasible matrix for extraction as the MLE bioactive compounds activity in the yogurt was not affected throughout 16 days of chilled storage. The present findings indicate that both yogurt samples made from cow's milk have antioxidant potentials that was influenced by casein haplotype due to specific amino acid sequence of the milk protein variants (de Carvalho et al. 2019). The detection of antioxidant activity in both samples (with and without MLE) was due to the bacterial fermentation which release several bioactive peptides, organic acid derivatives, phosphate, carotenoids and specific characteristic strain of LAB, which also contribute to the antioxidant activity (Perna et al. 2014; Alenisan et al. 2017; Khan et al. 2019). The higher antioxidant activity in yogurt with MLE was due to the presence of the phenolic contents in the MLE extracts as shown in Figure 2. Study by Ramírez et al. (2016) which also investigated on the antioxidant activity in mango leaf tea found the stable activity of antioxidant in the sample for up to 48 hours at 4℃. Zhao and Shah (2014) also found a stable antioxidant content in fermented soy milk with tea leaf (green and black) after 8 weeks of storage at 4℃.

Total phenolic content (TPC) in yogurt

As presented in Figure 4, it was clearly shown that there was no significant difference ($p>0.05$) between yogurt with and without mango leaf extract (MLE). However, throughout the storage, the total phenolic content (TPC) in yogurt with MLE remained until 8 days of storage time compared to the control sample and again the stable TPC during storage potentially due to the proteolysis of milk proteins which release amino acids with phenolic side chains (Raikos et al. 2019). In the current study, yogurt without supplementation or addition of MLE also tended to have antioxidant capacity which may due to the bioactive peptides through the proteolysis. In addition, the antioxidant content is also influenced by the heat treatment performed milk during the preparation of yogurt in which the production of organic acids was produced during the fermentation and post-acidification of yogurt due to aggregation of peptide processes that occur during enzymatic hydrolysis of whey protein and casein (Perna et al. 2014). However, the differences in total phenolic content values were observed for both yogurts in the presence and absence of MLE due to the degradation of the milk proteins from yogurt bacteria releasing phenolic acids and flavonoids that attached to milk protein. The trend shows that the longer the storage time, the lower the amount of phenolic content. This results is similar to a previous study by Cho et al. (2017) showed that the total value of phenolic content of plain yogurt and yogurt supplemented or added with green olives decreased over longer storage periods due to the phenolic disintegration of polymers with the presence of lactic acid bacteria in the yogurt during storage.

Aerobic Plate Count

There was no significant difference ($p>0.05$) was observed in yogurt with the presence or absence of MLE extract (Figure 5). This suggests that the

presence of MLE in yogurt does not significantly influence the growth rate of microorganisms present in the samples. The presence of LAB which have capabilities to produce metabolites such as organic acids, hydrogen peroxide, bacteriocins-like substance and diacetyl should reduce the growth of the spoilage organisms (Willer et al. 2011). However, in this study the trend showed for both yogurts with or without extract was an increase in the growth concentration of the spoilage bacteria when storage time was longer which can result in post-pasteurisation contamination which could have adverse effect in consumer's health (Moh et al. 2017).

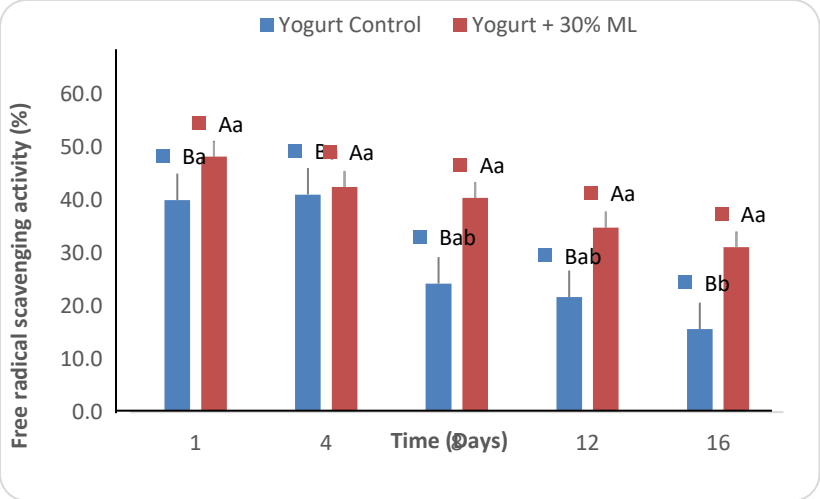

Figure 3: The Effects of incorporation of mango leaves extract and storage time on the free radical scavenging activity of the yogurt within 16 days storage. Result are presented as means ± SD. *Different letters (A-B) indicate significant difference (p<0.05) between samples. *Different letters (a-b) indicate significant different (p<0.05) between storage day

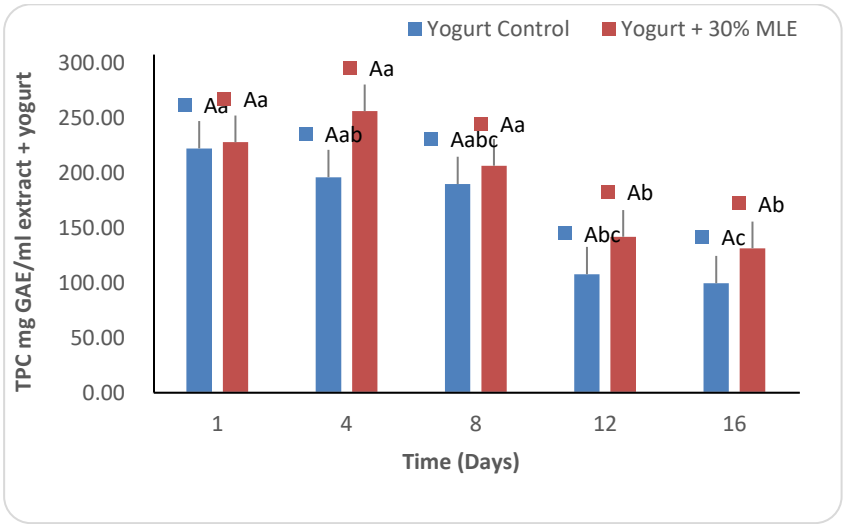

Figure 4: The Effects of incorporation of mango leaves extract and storage time on total phenolic content of the yogurt within 16 days storage. Result are presented as means ± SD. *Different letters (A-B) indicate significant difference (p<0.05) between samples. *Different letters (a-b)

indicate significant different (p<0.05) between storage day

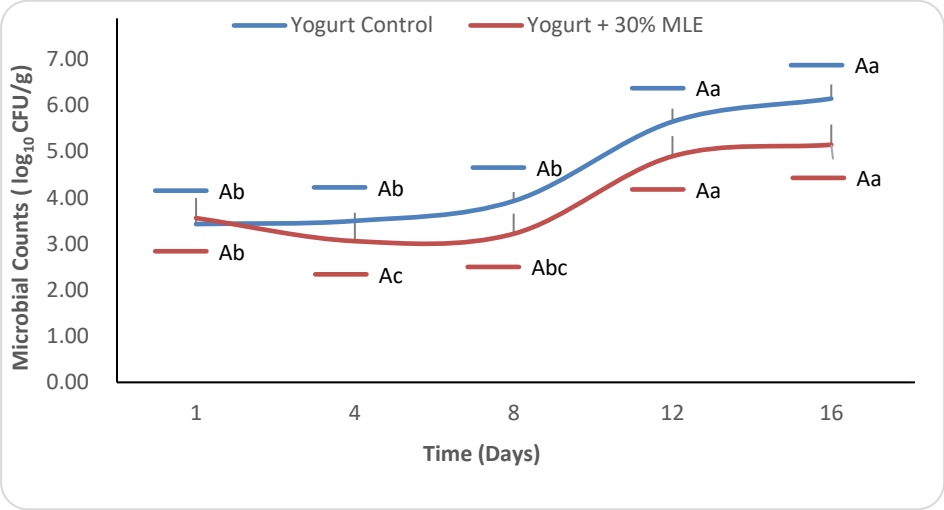

Figure 5. Effect of incorporation of mango leaves extract and storage time on the Aerobic Plate Count of yogurt within 16 days storage. Result are presented as means ± SD. *Letter (A) indicate no significant difference (p>0.05) between samples. *Different letters (a-c) indicate significant different (p<0.05) between storage day

Mold and Yeast Count

There was no significant difference (p>0.05) between yogurt incorporated with MLE and yogurt control (Figure 6). However, the trend of both yogurt samples showed that from day 8 onwards, the concentration of mould and yeast count decreased significantly (p<0.05) with storage time. The result also showed that the yeast and mould growth was stable from day 1 to day 8 which may be due to the optimal cell adhesion so that yeast and mould populations began to decline. This was potentially caused by the depleted nutrients and residues began to accumulate in both yogurt samples. However, the trend suggests that yogurt with the addition of MLE has a lower concentration of microbial count than yogurt control because mango leaves could potentially to be used as an antifungal agents and antimicrobial activities due to the presence of the phytochemical compounds (Disegha and Akani, 2019).

Viability of lactic acid bacteria (LAB)

LAB count on day 1 was 6.08-7.09 $\log_{10}$ CFU/g indicating that that there was growth of LAB in both of the yogurt throughout the storage time (Figure 7). There was a significant difference (p<0.05) for both yogurt samples during 16 days storage although no significant effects (p>0.05) was observed between yogurt samples with presence or absence of the extract in maintaining LAB. The significant increment of LAB at day 4 for treated yogurt indicating that phenolic compounds content in mango leaves played an important role and may enhance the growth of the starter cultures. Besides, polyphenols that present in mango leaves cause LAB to adapt and replicate. According to Sung et al. (2015) reported that the growth of LAB also can be influenced by vitamins, minerals and carbohydrates content. This means that LAB has the ability to survive under certain circumstances. However, the viability of LAB degraded during storage. This is not surprising since Najarian (2010) has reported that degradation of cell viability was associated with a decrease in pH during storage due to the accumulation of organic acids as the result of growth during fermentation. The decline in the LAB count or volume during storage may be due to extrinsic and intrinsic factors such as pH, acidity and temperature which related to LAB adaptation (Othaman et al. 2012).

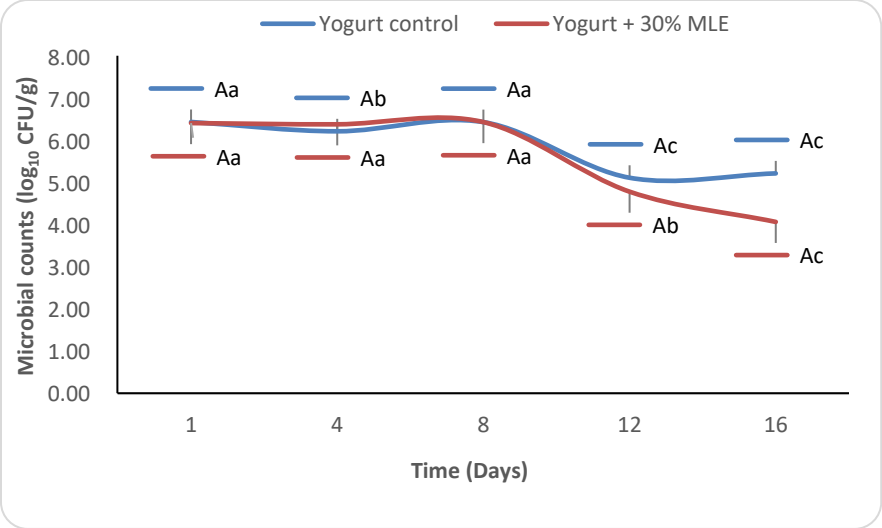

Figure 6: Effect of incorporation of mango leaves extract and storage time on mould and yeast of yogurt within 16 days storage. Result are presented as means ± SD. *Letter (A) indicate no significant difference (p>0.05) between samples. *Different letters (a-c) indicate significant different (p<0.05) between storage day

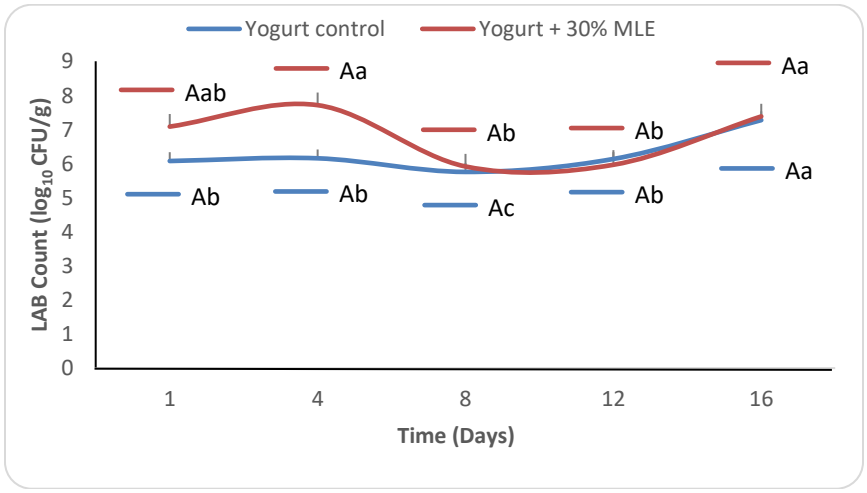

Figure 7: Effect of incorporation of mango leaves extract and storage time on the viability of LAB of yogurt within 16 days storage. Result are presented as means ± SD. *Letter (A) indicate no significant difference (p>0.05) between samples. *Different letters (a-c) indicate significant different (p<0.05) between storage day

pH

Incorporation of MLE into yogurt gave no effect on the pH value of the product. There was a declining value for both treatments ($p<0.05$) during 16 days of storage. The declined value of pH in yogurt with MLE at the end of storage indicating the higher fermentation occurred. This means that the viability of LAB was affected by MLE when the pH value is low due to the high production of lactic acid by LAB (Shima et al. 2012).

Titratable acidity

Figure 9 shows the rate of acid formation was significant ($p<0.05$) between all samples indicating the effect of MLE on the total acidity in yogurt. The acidity of the yogurt also increased with the storage time. The increment of acidity during storage was potentially due to the transformation of lactose into lactic acid by LAB n. which leads to an increase in acidity (Mamoona et al. 2013). This result is compatible with those reported by Nguyen et al. (2016) who reported that during storage, the quantity of acidity increased due to the amount of the lactic acid found in the yogurt and was almost linear compared to the pH change. However, based on the LAB count in Figure 7, the trends for the viability of LAB showed the highest amount of LAB on day 4 then decreased on day 8 and then started to rise again until end of storage while the mean value for titratable acidity was slightly increased constantly for the whole storage. The accumulation of acid may not only contributed by LAB but also acetic acid, citric acid, butyric acid, acetaldehyde and formic acid as the metabolic by-products of the fermentation (Shori, 2013). Thus, the higher acidity in yogurt with MLE indicated the result of higher organic acid as the by-product of better fermentatio

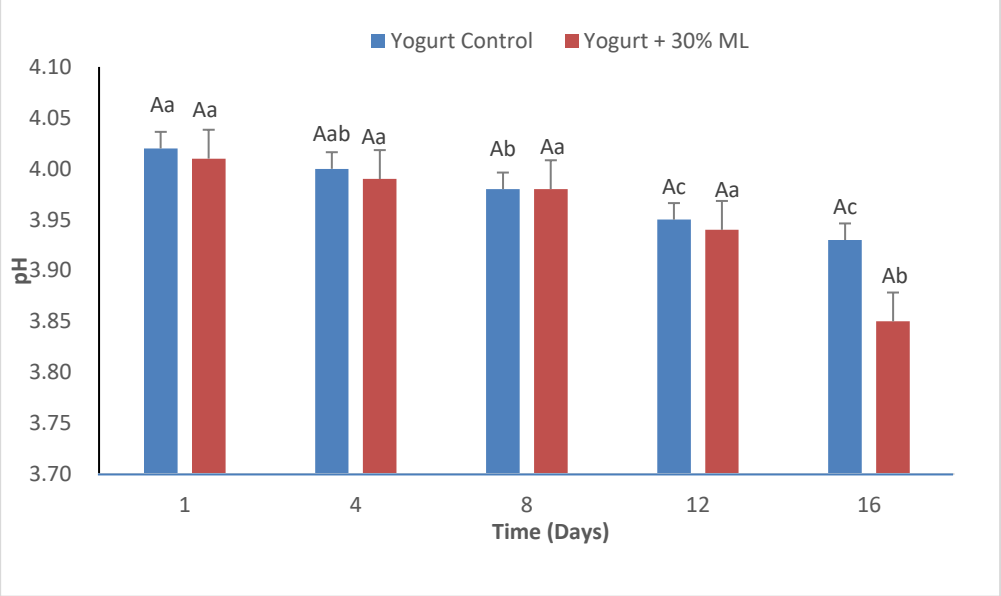

Figure 8: Effect of incorporation of mango leaves extract and storage time on the pH value of yogurt within 16 days storage. Result are presented as means ± SD. *Letter (A) indicate no significant difference ($p>0.05$) between samples. *Different letters (a-c) indicate significant different ($p<0.05$) between storage day

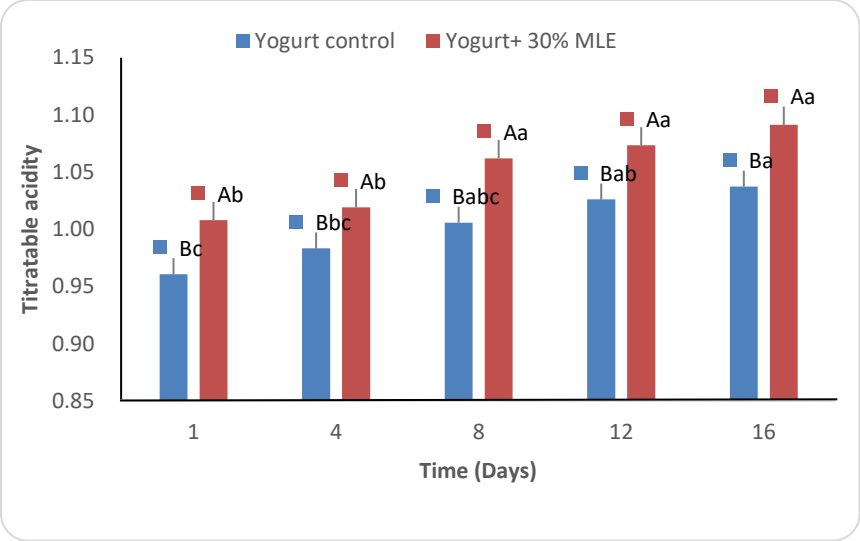

Figure 9: Effect of incorporation of mango leaves extract and storage time on the titratable acidity of yogurt within 16 days storage. Result are presented as means ± SD. *Letter (A) indicate no significant difference (p>0.05) between samples. *Different letters (a-c) indicate significant different (p<0.05) between storage day

CONCLUSION

This study showed that the incorporation of antimicrobial and antioxidant properties from the natural source such as mango leaf extract (MLE) enhanced the yogurt quality. The addition of 30% of MLE not only increased the antioxidant properties of yogurt but also reduce its degradation during storage. The viability count of lactic acid bacteria was also not affected with the addition of MLE and maintained at the recommended concentration (6 $\log_{10}$ CFU/g) during storage and showed reduction trend for mold and yeast count. It is suggested for future reference, a research should be done on the proximate and organoleptic properties of yogurt supplemented with MLE in order to improve the nutritional quality of commercial yogurt.

ACKNOWLEDGMENTS

We thank the Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu for providing the funding and technical supports in this research.

REFERENCES

Alenisan MA, Alqattan HH, Tolbah LS, Shori A B. 2017. Antioxidant properties of dairy products fortified with natural additives: A review. *Journal of the Association of Arab Universities for Basic and Applied Sciences*, 24(1): 101-106.

Bagchi K, Puri S, 1998. Free radicals and antioxidants in health and disease: A review. *Eastern Mediterranean Health Journal*, 4 (2): 350-360

Cho WY, Yeon SJ, Hong GE, Kim JH, Tsend-Ayush C, Lee CH 2017. Antioxidant activity and quality characteristics of yogurt added green olive powder during storage. *Korean Journal for Food Science of Animal Resources* 37(6): 865.

de Carvalho MW, Arriola NDA, Pinto SS, Verruck S, Fritzen-Freire CB, Prudêncio ES, Amboni RDDMC, 2019. Stevia-fortified yoghurt: Stability, antioxidant activity and in vitro digestion behaviour. *International Journal of Dairy Technology* 72(1): 57- 64

Disegha GC, Akani N, 2019. Antifungal activity of *Mangifera indica* leaf extracts on selected fungi. *Current Studies in Comparative Education, Science and Technology* 7(4): 46-58.

Fernández-Ponce MT, Parjikolaei BR, Lari HN, Casas L, Mantell C, de la Ossa EJM, 2016. Pilot-plant scale extraction of phenolic compounds from mango leaves using different green techniques: Kinetic and scale up study. *Chemical Engineering Journal* 299(16): 420-430.

Gied AAA, Joseph MR, Mahmoud IM, Abdelkareem AM, Al Hakami AM, Hamid ME, 2012. Antimicrobial activities of seed extracts of mango (*Mangifera indica* L.). *Advances in Microbiology* 2(04): 571-576.

Hannan A, Asghar S, Naeem T, Ullah MI, Ahmed I, Aneela S, Hussain S, 2013. Antibacterial effect of mango (*Mangifera indica* Linn.) leaf extract against antibiotic sensitive and multi-drug resistant *Salmonella* typhi. *Pakistan Journal of Pharmaceutical Sciences* 26(4): 715-719.

Harvey RA, Champe PC, Fisher BD, 2007. Microbiology. Philadelphia. Lippincot Williams and Wilkins.

Ismail A, Marjan ZM, Foong CW, 2004. Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry Journal* 87(4): 581-586.

Kalumbi M, 2018. Effect of processing method and concentration of *Mangifera indica* leaf extract on their antibacterial activity against *Staphylococcus aureus. International Journal of Herbal Medicine* 6(6): 116-119.

Khan IT, Nadeem M, Imran M, Ullah R, Ajmal M, Jaspal MH, 2019. Antioxidant properties of Milk and dairy products: a comprehensive review of the current knowledge. *Lipids in health and disease* 18(1): 41-50.

Kingne FK, Tsafack HD, Boungo GT, Mboukap A, Azia A, 2018. Phenolic Content and Antioxidant Activity of Young and Mature Mango (*Mangifera indica*) and Avocado (*Persea americana*) Leaves Extracts. *Journal Food Stability* 18(1): 14-27.

Kulkarni V, Rathod V, 2016. Green process for extraction of mangiferin from *Mangifera indica* leaves. *Journal of Biologically Active Products from Nature* 6(5): 406-411.

Lani MN, Adnan NA, Nurmahani MM, Ibrahim R, Hassan Z, 2019. Microbiological quality and sensory evaluation of partially dried mango for fruit salad, Kerabu Mangga. *Asian Journal of Agriculture and Biology* 7(1): 103-115.

Lani MN, Zainudin AH, Razak SBA, Mansor A, Hassan Z, 2017. Microbiological quality and pH changes of honey produced by stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica* stored at ambient temperature. *Asian Journal of Agriculture and Biology* 7(1): 103-115.

Lestari RP, Nissa C, Afifah DN, Anjani G, Rustanti N, 2018. Total Lactic Acid Bacteria (LAB), Antioxidant Activity, and Acceptance of Synbiotic Yoghurt with Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis). In *IOP Conference Series: Earth and Environmental Science Journal*. 116(1): 1-6.

Lin SD, Liu EH, Mau JL, 2008. Effect of different brewing methods on antioxidant properties of steaming green tea. *Learning with Technologies- Food Science and Technology* 41(9), 1616-1623.

Luo F, Lv Q, Zhao Y, Hu G, Huang G, Zhang J, Chen K, 2012. Quantification and purification of mangiferin from Chinese mango (*Mangifera indica* L.) cultivars and its protective effect on human umbilical vein endothelial cells under H_2O_2-induced stress. *International Journal of Molecular Sciences* 13(9): 11260-11274.

Mamoona A, Sarfraz A, Aysha S, Saeed A. 2013. Effect of different heating temperatures on the rheological properties of lactic gel made from buffalo milk. *Journal of Food Chemistry and Nutrition* 1(1): 33-41

Michael M, Phebus RK, Schmidt KA, 2015. Plant extract enhances the viability of *Lactobacillus delbrueckii subsp. bulgaricus* and *Lactobacillus acidophilus* in probiotic nonfat yogurt. *Journal of Food Science & Nutrition* 3(1): 48-55.

Moh LG, Keilah LP, Etienne, PT, Jules-Roger, K., 2017. Seasonal Microbial Conditions of Locally Made Yoghurt (Shalom) Marketed in Some Regions of Cameroon. *International Journal of Food Science* 17(1): 1-16.

Najarian A, 2010. Changes in microbiological, physicochemical, biochemical and therapeutical characteristics of yoghurt upon the addition of selected medicinal herbs. Master thesis, University of Malaya. 1-141.

Nguyen L, Hwang ES, 2016. Quality characteristics and antioxidant activity of yogurt supplemented with aronia (Aronia melanocarpa) juice. *Preventive Nutrition and Food Science* 21(4): 330.

Olasehinde GI, Sholotan KJ, Openibo JO, Taiwo, OS, Bello OA, Ajayi JB, Ajayi AA, 2018. Phytochemical and antimicrobial properties of *Mangifera indica* leaf extracts. *Covenant Journal of Physical and Life Sciences* 6(1): 55-63.

Othaman SF, Aziah AA, Ahmad R, 2012. Effects of jackfruit puree supplementation on lactic acid bacteria (*Lactobacillus acidophilus* FTDC 1295) in terms of viability and

chemical compositions of dadih. *International Food Research Journal* 19(4): 1687-1697.

Perilla MJ, 2003. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world; *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae, Neisseria gonorrhoeae, Salmonella* serotype *Typhi, Shigella, and Vibrio cholerae.*

Perna A, Intaglietta I, Simonetti A, Gambacorta E, 2014. Antioxidant activity of yogurt made from milk characterized by different casein haplotypes and fortified with chestnut and sulla honeys. *Journal of Dairy Science* 97(11): 6662-6670.

Qian Y, Long X, Pan Y, Li G, Zhao X, 2018. Isolation and identification of lactic acid bacteria (*Lactobacillus plantarum* YS2) from yak yogurt and its probiotic properties. *Biomedical Research* 29(4): 815-820.

Raikos V, Ni H, Hayes H, Ranawana V, 2019. Antioxidant properties of a yogurt beverage enriched with Salal (*Gaultheria shallon*) berries and blackcurrant (*Ribes nigrum*) pomace during cold storage. *Beverages* 5(1): 2-9.

Ramírez NM, Farias LM, Santana FA, Leite JPV, Dantas MIDS, Toledo RCL, Ribeiro SMR, 2016. Extraction of mangiferin and chemical characterization and sensorial analysis of teas from *Mangifera indica* L. leaves of the ubá variety. *Beverages* 2(4): 1-8.

Seo J, Lee S, Elam ML, Johnson SA, Kang J, Arjmandi BH, 2014. Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Science & Nutrition* 2(2): 174-180.

Salleh F, Lani MN, Tuan Chilek TZ, Kamaruding NA, Ismail N, 2021. Lactic acid bacteria producing sorbic acid and benzoic acid compounds from fermented durian flesh (Tempoyak) and their antibacterial activities against foodborne pathogenic bacteria. *Applied Food Biotechnology* 8(2): 121-132.

Shafiee G, Mortazavian AM, Mohammadifar M A, Koushki MR,,Mohammadi A, Mohammadi, R, 2010. Combined effects of dry matter content, incubation temperature and final pH of fermentation on biochemical and microbiological characteristics of probiotic fermented milk. *African Journal of Microbiology Research* 4(12): 1265-1274.

Shah KA, Patel MB, Patel RJ, Parmar PK, 2010. *Mangifera indica* mango). *Pharmacognosy reviews* 4(7): 42.

Sharma R, Sanodiya BS, Bagrodia D, Pandey M, Sharma A, Bisen PS. 2012. Efficacy and potential of lactic acid bacteria modulating human health. *International Journal of Pharma and Bio Sciences* 3(4): 935-948.

Sherman C, 2011. Microbiology a laboratory manual. San Francisco: Benjamin Cummins.

Shima AR,Salina HF, Masniza M, Atiqah AH, 2012. Viability of lactic acid bacteria in homemade yogurt containing sago starch oligosaccharides. *International Journal Basic and Applied Science* 12(1): 58-62.

Shori AB, 2013. Antioxidant activity and viability of lactic acid bacteria in soybean-yogurt made from cow and camel milk. *Journal of Taibah University for Science* 7(4): 202-208.

Sung JM, Kim YB, Kum JS, Choi YS, Seo DH, Choi, HW, Park JD, 2015. Effects of freeze-dried mulberry on antioxidant activities and fermented characteristics of yogurt during refrigerated storage. *Korean Journal for Food Science of Animal Resources* 35(6): 807.

Tomovska J, Gjorgievski N, Makarijoski B, 2016. Examination of pH, titratable acidity and antioxidant activity in fermented milk. *Journal of Materials Science and Engineering Agriculture* 16(3): 326-333.

Willey JM, Sherwood LM, Woolverton CJ, 2011. Epidemiology and public health microbiology:Nosocomial infections. Willey JM, Sherwood LM and Woolverton CJ. edited Prescott's Microbiology. International Edition. 8(11), 873-884.

Zhao D, Shah NP, 2014. Antiradical and tea polyphenol-stabilizing ability of functional fermented soymilk–tea beverage. *Food Chemistry* 158 (2): 262–26