

Effect of incorporation of honey on chilled storage and sensory acceptance of probiotic Melon Manis Terengganu (*Cucumis melo var inodorus cv. Manis Terengganu 1*) juice

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Abstract

The objective of this study was to determine the effect of 28 days of chilled storage (4°C) on Melon Manis Terengganu (MMT) juice, probiotic MMT juice and probiotic MMT juice with 3.9% honey. Furthermore, determination of sensory evaluation of these three juices using acceptance test was carried out as well. It was found that for all MMT juice samples with 28 days of chilled storage resulted in increased total soluble solid, decreased viscosity and no significant difference in titratable acidity. Besides, the pH of control MMT juice did not change, but the pH of both probiotic MMT juices decreased. It was found that there was no yeast and mould growth in probiotic MMT juice with 3.9% honey until the 28th-day storage. However, yeast and mould growth appeared after 21 days of chilled storage for both control MMT juice and probiotic MMT juice, with control MMT juice having the higher yeast and mould count. During chilled storage, there was no LAB count and no significant change in biomass in control MMT juice, but there was a decreasing trend for LAB count and biomass for probiotic MMT juice after 21 days of storage. However, for probiotic MMT juice with honey, LAB count did not change significantly, while its biomass was higher than probiotic MMT juice after 14 days of chilled storage. Sensory evaluation found that there was no significant difference ($p < 0.05$) in acceptability of all MMT juice samples for sourness, overall acceptability and odour attributes. For colour attribute, probiotic MMT juice gave similar colour attribute acceptance with other samples. However, both probiotic juices gave lower acceptability scores for flavour and sweetness attributes compared to control MMT juice. This study found that the most acceptable formulation was control MMT juice, followed by MMT probiotic juice and finally MMT probiotic juice with 3.9% honey.

1. Introduction

Fruit juice is a natural liquid contained in ripe fruits and rich in beneficial nutrients such as sugar, vitamins, minerals, fibre, and antioxidants. Thus, fruit juice is suitable substrates for the probiotic bacteria cultivation. Many researchers have been investigated the suitability of various fruit and vegetable juices as raw material for the production of probiotic juices such as tomato, mango, orange, apple, grape, peach, pomegranate and watermelon (Patel, 2017). Furthermore, fruit juices are highly prone to deterioration and the short shelf life of the fresh juices usually are caused by enzymatic, microbial, and chemical factors (Ephrem *et al.*, 2018). According to Lebaka *et al.* (2018), the physicochemical and sensory qualities, especially flavour and nutritional components can be modified by fermentation.

Probiotics are defined as microorganisms that have a positive effect on the intestinal of the host by balancing the intestinal flora (Fuller, 1989). The minimum requirement for the number of probiotics bacteria present in the beverages is 10^7 CFU/g during consumption time (Angiolillo *et al.*, 2014). The health advantage of probiotics chiefly depends upon their concentration in foods and on their ability to endure the unfavourable conditions of the gastrointestinal tract. The major parameters influencing the number of probiotics are intrinsic food parameters such as titratable acidity, pH, sugar, molecular oxygen and water activity. Incubation temperature, storage techniques, kind of probiotics strains and inoculums proportion also influence the growth of probiotics bacteria (Tripathi and Giri, 2014). Among the most commonly employed probiotics are *Lactobacillus acidophilus*, *L. helveticus*, *L. casei*, *L.*

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paracasei, *L. johnsonii*, *L. plantarum*, *B. bifidum* and *B. longum* (Nagpal *et al.*, 2012; Patel, 2017). The non-dairy probiotic foods market is projected to have an annual growth rate of 15% between 2013 and 2018 (Ephrem *et al.*, 2018). Martins *et al.* (2013) found that fruits, such as apple, guava, banana, and melon, have potential as carriers for probiotics bacteria.

Honey is a natural sweetener that can act as potential prebiotic because it contains many oligosaccharides and low molecular weight polysaccharides which are neither hydrolysed by host enzymes or absorbed in the upper part of the gastrointestinal tract (Hirschberg *et al.*, 2006). It can remain available as a nutrient source for the intestinal microflora thereby acknowledged as a prebiotic material (Jan Mei *et al.*, 2010). Sanz *et al.* (2005) reported honey oligosaccharides to provide potential prebiotic activity by increasing the populations of faecal *bifidobacteria* and *lactobacilli*. Most of the honey incorporated beverages were reported in the dairy product. Besides that, the natural present of *Lactobacillus* species in the honey can help to inhibit the growth of pathogens and bring advantageous properties for honey (Forsgren *et al.*, 2010). In addition, the antibacterial components found in honey can enhance the probiotic efficacy against pathogens (Mohan *et al.*, 2017). Machado *et al.* (2017) and Nath *et al.* (2015) found that the overall acceptability of honey incorporated probiotic beverages was greater than those without added honey.

Melon Manis Terengganu (*Cucumis melo var inodorus cv. Manis Terengganu 1*) is one of the melon species under Cantaloupe family. It has a smooth yellow-golden without netted beige of skin and together with crunchy orange coloured flesh (Muhamad *et al.*, 2018). Fresh melon juice contains considerable amounts of total soluble solids, total sugars, reducing sugars, ascorbic acid, minerals, and free amino acids. Development of value-added products from MMT is needed because sometimes there is an oversupply of MMT fruit and rejected MMT fruit which is not suitable to be sold as whole fruit. This will help to reduce waste and generate value-added products from MMT industry. MMT juice is one of the products that can be developed from MMT. To date, no study has been reported on the effect of honey and probiotics bacteria on the changes during chilled storage and its sensory acceptance. This MMT juice also may offer alternative non-dairy probiotic juice to lactose-intolerant consumers. Thus, the objective of this study was to determine the effect of chilled storage on lactic acid bacteria (LAB) growth and physical properties of MMT juice (control), probiotic MMT juice without honey and probiotic MMT juice with 3.9% honey as well as to determine the sensory acceptance of

these three juices.

2. Materials and methods

2.1 Raw materials

A total of 20 kg Melon Manis Terengganu and 1 kg of Tualang honey were purchased from a supplier in Gong Badak, Terengganu. Melon Manis Terengganu was stored in the freezer at -20°C for further used. All other chemical reagents used were of analytical grades.

2.2 Sample preparation

The whole fresh MMT fruits were peeled and deseeded and the flesh was cut into small pieces. After that, it was blended into juice by using a blender (Pensonic, Malaysia). The juice was then stored in -20°C for further use.

2.3 Inoculum preparation

Pure culture of *Lactobacillus plantarum* was cultured in a 250 mL Erlenmeyer flask containing 100 mL of MRS broth for 24 hrs at 37°C (Puertollano *et al.*, 2009). The culture was transferred into 50 mL centrifuge tubes and centrifuged at 10 000 rpm for 10 mins at 4°C (Ricci *et al.*, 2019). The supernatant produced was discarded. The pellet was washed with phosphate buffer saline (PBS) (pH 7.3). The mixture of PBS and pellet was centrifuged once again at 10 000 rpm for 10 mins at 4°C. The mixture was then washed again with PBS to be used as inoculum. The purity of the culture was checked by streaking on MRS agar. Single colony on MRS agar indicated pure culture. The cell density was determined spectrophotometrically at 590 nm (GENESYS 20 Thermospectronic, Thermo Electron Corp., USA) until it reached 0.600 that correspond to 9.00 log CFU/mL, using MacFarland scale (Pereira *et al.*, 2011). This culture was used as a probiotic inoculum for juice fermentation.

2.4 Effect of honey on chilled storage of probiotic MMT juice

MMT juice was prepared by pasteurizing the thawed juice at 80°C for 3 mins to kill the harmful microorganisms (Kaya *et al.*, 2015). Next, MMT juice (control) was dispensed into glass jars and stored for chilled storage study for 28 days at 4°C.

To prepare probiotic juices, 100 mL of pasteurized MMT juice in Erlenmeyer flasks were inoculated with 7.00 log CFU/mL *L. plantarum* (1 mL of inoculum containing 9.00 CFU/mL of *L. plantarum*). This concentration was chosen based on the recommendation for probiotic foods which should have a minimum count of 7.00 log CFU/mL for better efficacy in regulating

beneficial effects (Vinderola and Reinheimer, 2000). Fermentation of 2 types of MMT probiotic juices (without honey and with 3.9% honey) were carried out statically in an incubator for 24 hrs at pH 5.5, 38°C. Then, both types of MMT probiotic juices were dispensed into glass jars and stored for chilled storage study at 4°C for 28 days. Each MMT juice sample was taken every 7 days interval and was subjected to determination of biomass, LAB cell count, yeast and mould count, pH, colour, titratable acidity, viscosity, and total soluble solids.

2.4.1 Biomass determination

The MMT juices were diluted with distilled water (1:10). Then, the biomass was determined by optical density measured at 590 nm (GENESYS 20 Thermospectronic, Thermo Electron Corp., USA) (Rodrigues *et al.*, 2003). The distilled water was used as blank. Biomass was calculated based on a calibration curve.

2.4.2 Viable LAB counts

To determine the viability of lactic acid bacteria (LAB), deMan, Rogosa and Sharpe (MRS) agar was used. Serial dilutions of probiotic MMT juice sample with sterile peptone water were prepared until 10^{-6} dilution. Then, 0.1 mL of the sample was plated using spread plate method. The plates were then incubated for 72 hrs at 37°C. Plates containing 20-350 colonies were counted and LAB counts were calculated as colony forming units (CFU) per mL of solution. According to Vinderola and Reinheimer (2000), LAB has characteristics round white creamy colonies with diameters from 0.9 to 1.3 mm.

2.4.3 Yeast and mould count

Dichloran Rose Bengal Chloramphenicol (DRBC) agar was used to determine the yeast and mould count. Serial dilutions of the sample with sterile peptone water were prepared until 10^{-3} dilution. Then, 0.1 mL of the sample was plated using a spread plate method. The plates were incubated for 5 days at 30°C. Plates containing 20-350 colonies were counted. Yeast and mould count were stated as colony forming units (CFU) per mL of solution.

2.4.4 Determination of pH

pH of the probiotic MMT juice was determined by using a pH meter (Eutech Instruments Pte. Ltd., Singapore).

2.4.5 Colour analysis

Colour analysis was determined using a

chromameter (Konica Minolta Inc., Japan). After calibration of the chromameter, the MMT juice was placed in a transparent container and its L*, a*, b* values were measured.

2.4.6 Titratable acidity

Six grams of each juice sample was weighed into a 100 ml beaker. Then, 50 ml of distilled water and several drops of phenolphthalein were added into each sample. Each sample was titrated with 0.1N NaOH to the endpoint. The volume of NaOH used for titration was recorded.

2.4.7 Viscosity

Viscosity of the juice samples was measured using a viscometer (Brookfield DV-11+, Middleboro, MA, USA). Spindle 2 was used to determine the viscosity of beverage at a speed of 100 rpm.

2.4.8 Determination of total soluble solids

Total soluble solids (TSS) is equal to the percentage of sugar and other soluble or dissolved solids in a solution. A digital refractometer (model MA871, Milwaukee Instruments Inc, USA) was used to determine the TSS of the samples as °Brix. Distilled water was used as a blank solution.

2.5 Sensory evaluation

Sensory evaluation for control MMT juices, probiotic MMT juices without honey and probiotic MMT juices with 3.9% honey were also carried out using an acceptance test. A 7-point hedonic scale (1=dislike extremely to 7=like extremely) were used to evaluate the acceptance of the products. The sensory attributes including the colour, odor, Melon Manis Terengganu flavour, sweetness, sourness, overall acceptability were evaluated by forty untrained panels. All the samples were served chilled. Samples were served in paper cups and were labelled with 3-digit code.

2.6 Statistical analysis

All data were presented as mean±standard deviation. Minitab 14 software (Minitab Inc., USA) was used to conduct statistical analysis for all the results obtained from analysis and sensory evaluation. One-way analysis of variance (ANOVA) was used to determine the significant difference between the juice samples as well as the storage time. Results with significant differences were further analysed by using Tukey's comparison test to determine the significant difference among the independent variables ($p < 0.05$).

3. Results and discussion

3.1 Chilled storage study

3.1.1 Biomass

Biomass of LAB was measured by using a spectrophotometer. As the LAB population increased, the amount of transmitted light decreased due to the increasing turbidity. Thus, the absorbance reading reflects the number of bacteria. Figure 1 shows that the effect of incorporation of honey and LAB on the biomass of MMT juice within 28 days of chilled storage time. There was a significant difference between the biomass of the control MMT juice and the probiotic MMT juice with and without honey ($p < 0.05$). For each storage time interval, control MMT juice always gave lower biomass than both probiotic MMT juices due to the presence of LAB in the probiotic MMT juices. There was no significant difference between the biomass for both probiotic MMT juices ($p > 0.05$). The presence of biomass in the control MMT juice may be due to the presence of pomace in the fruit juice which influences the amount of transmitted light pass through the juice.

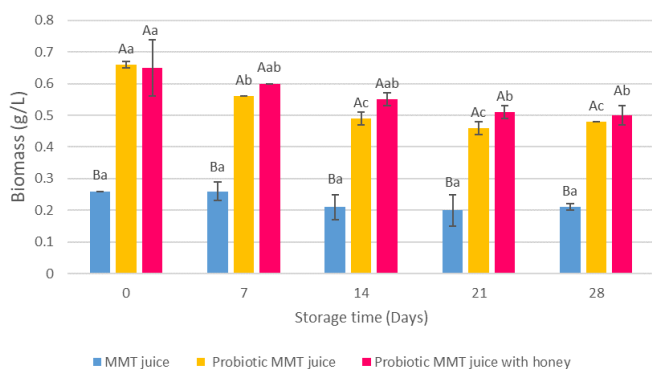


Figure 1. Effect of incorporation of honey and LAB on biomass of MMT juice within 28 days of chilled storage time. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

During the 28 days of chilled storage, as expected, there was no significant difference ($p > 0.05$) in the biomass of the control MMT juice. On the other hand, both probiotic MMT juice with and without honey showed a significant decrease ($p < 0.05$) in biomass. This may be due to the decrease in the number of LAB because of the sugar content in the MMT juice was unable to support the growth of the LAB. The reduction of the number of LAB caused the reduction of biomass. However, Pereira *et al.* (2011) reported a different finding whereby there was an increase in the biomass of the probiotic cashew apple juice fermented by *L. casei*, but it was not followed by the decrease in viability of probiotic cells. They reported that these results indicated

that some cells lost their viability at the end of storage although biomass in the juice remained high.

3.1.2 LAB count

LAB count is important to determine the amount of probiotic strain present in the fermented drinks. A probiotic drink should contain at least 6 - 7 log CFU/g or mL of viable probiotic bacteria at the time of ingestion to promote health benefits to the consumers (Vinderola *et al.*, 2000). Figure 2 shows the effect of incorporation of honey and LAB on LAB cell count of MMT juice within 28 days of chilled storage time. As expected, there was no LAB growth found in the control MMT juice throughout the 28 days chilled storage. It was found that there was no significant difference in LAB count of both probiotic MMT juices on 0, 7th and 14th day ($p > 0.05$). However, a significant decrease was found in LAB count on 21st and 28th day of storage. The LAB count of probiotic MMT juice without honey dropped from 7.58 log CFU/mL to 6.77 log CFU/mL at the 21st day and decreased further to 5.88 log CFU/mL at the end of the chilled storage time. This may be due to the insufficient sugar content in MMT juice to support LAB growth.

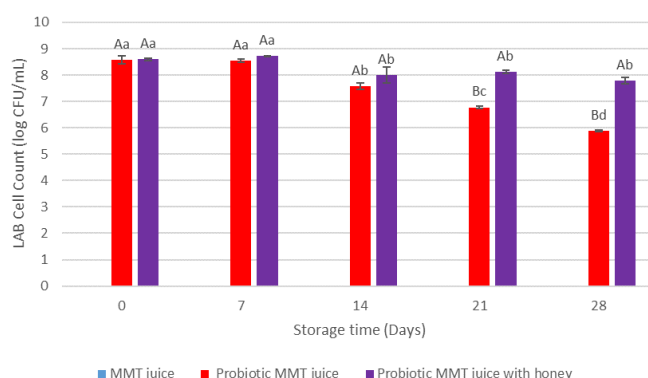


Figure 2. Effect of incorporation of honey and LAB on LAB cell count of MMT juice within 28 days of chilled storage time. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

It was also noted that probiotic MMT juice with honey gave higher LAB count than that of without honey on day-21 and onwards. Honey contains a variety of oligosaccharides varying in degree of polymerization which can enhance the growth, activity and viability of *lactobacilli* and *bifidobacteria* fermented dairy and non-dairy products (Slaćanac *et al.*, 2011). The natural presence of *Lactobacillus* species in the honey can also enhance the number of probiotic in MMT juice (Forsgren *et al.*, 2010). Jan Mei *et al.* (2010) also reported that the addition of stingless bee honey in yoghurts appeared to assure the maintenance of higher counts in comparison to

the formulation without added honey over time.

For the same juice sample during 28 days of chilled storage, LAB count of probiotic MMT juice without honey decreased gradually after day-14 onwards. LAB count of probiotic MMT juice with honey also decreased after day-14. However, the LAB count in probiotic MMT juice with honey remained constant until the end of storage (7.78 log CFU/mL). This finding indicates that the carbohydrate content in the MMT juice was not enough to support the growth of the LAB.

This result was different to the finding of Pereira *et al.* (2012) who reported that the viable LAB count of probiotic cashew apple juice increased from 8.31 log CFU/mL to 8.63 log CFU/mL at the end of 28th day of storage at 4°C. Riazi and Ziar (2012) also observed the protective effect of honey on the cell viability of LAB in fermented skim milk during 28 days of refrigerated storage.

3.1.3 Yeast and mould count

Yeast and mould count is important to detect and quantify the amount of fungal growth on food as well as allow for the identification of viable yeast and mould species present. Figure 3 shows the effect of incorporation of honey and LAB on yeast and mould count of MMT juice within 28 days of chilled storage time. For the first 14 days of chilled storage, all samples did not show any growth of yeast and mould. On day-21 onwards, control juice gave higher yeast and mould count than probiotic MMT juice without honey ($p < 0.05$). Probiotic MMT juice with honey showed no growth of yeast and mould throughout 28 days chilled storage. This may be due to high LAB count of 7.78 log CFU/mL at the end of storage, and the LAB presence can help to inhibit the growth of the yeast and mould.

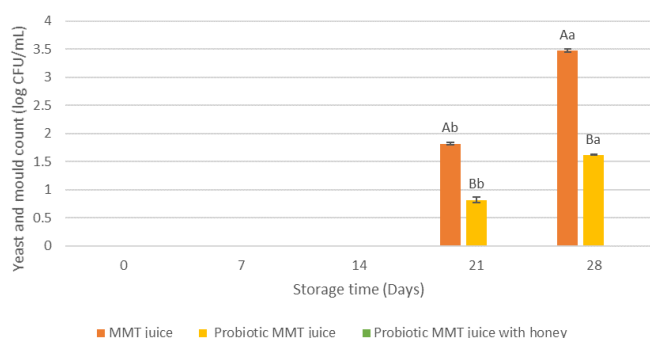


Figure 3. Effect of incorporation of honey and LAB on yeast and mould count of MMT juice within 28 days of chilled storage time. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

One of the functional requirements of LAB is its

antagonistic activity against pathogens. The mechanisms of probiotic to protect food against pathogen include lowering of pH, antagonism by producing bacteriocins which can kill pathogens and compete with pathogens for binding sites and receptors sites, nutrients and growth factors (Kosgey *et al.*, 2019). Bazukyan *et al.* (2018) also found the antifungal activity of lactobacilli isolated from Armenian dairy products, whereby the LAB produce bacteriocin-like compounds, organic acids can inhibit the growth of mould and thereby preventing aflatoxin B1 production.

There was a significant difference in yeast and mould count of control MMT juice sample and probiotic MMT juice without honey from 21st day onwards ($p < 0.05$). For control MMT juice, yeast and mould count was 1.82 log CFU/ (on day-21) and increased to 3.48 log CFU/mL at end of the day of chilled storage. Yeasts and moulds often use fruit juice as substrate and cause its spoilage by producing off-flavours, off-odours and discolouration (Tournas *et al.*, 2006). Tarazona-Díaz *et al.* (2017) reported that the yeast and mould count of pasteurised watermelon (80°C, 90 s) was above 3.00 log CFU/mL on day-15 of chilled storage. For the probiotic MMT juice without honey, it was less than 1 log CFU/mL initially and increased to 1.62 log CFU/mL at the end of chilled storage. The increase of yeast and mould count may be due to low pH condition was suitable for yeast and mould growth and the reduction of probiotic microorganisms resulted in the reduction of antifungal effect. According to the USDA (2001), yeast and mould count should not more than 50 per ml. Thus, both probiotic MMT juices were still safe after 28 days of chilled storage. For control MMT juice, its yeast and mould count exceed the USDA limit on day-21 onwards.

3.1.4 pH analysis

pH is an important factor that affects the viability of LAB and sensory attribute of the probiotic fruit juice. Figure 4 shows the effect of incorporation of honey and LAB on pH of MMT juice within 28 days of chilled storage time. There was a significant difference in pH between control MMT juice and both probiotic MMT juices ($p < 0.05$). The pHs of both probiotic MMT juices were lower than that of control MMT juice. This was due to the presence of LAB which utilized the sugar present in the juice and caused accumulation of lactic acid. There was no difference in pH between both probiotic MMT juices except on day-21 and day-28. Probiotic MMT juice with honey on day-28 gave lower pH probably due to the addition of honey may cause a slight reduction of the initial pH. The acidity of honey came from naturally occurring organic acids in its composition (Chuttong *et al.*, 2016).

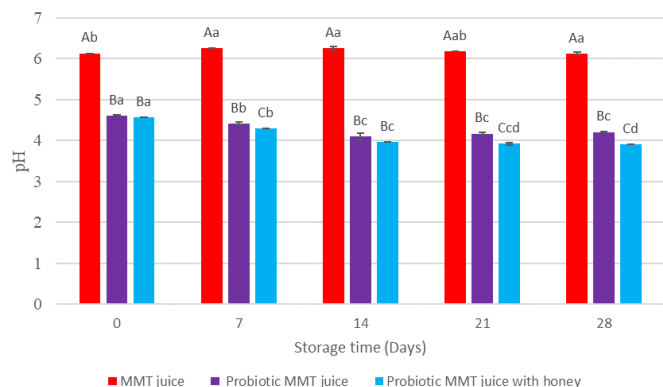


Figure 4. Effect of incorporation of honey and LAB on pH of MMT juice within 28 days of storage. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

The pH of control MMT juice did not change significantly during the 28 days of chilled storage. However, the pH of both probiotic MMT juices showed a significant decrease after 7 days of chilled storage. This is due to the fermentation of the glucose into lactic acid by the LAB. The pH range of probiotic MMT juice with and without honey obtained in this study were 4.03-4.62 and 3.89-4.57, respectively.

Pereira *et al.* (2011) reported a similar trend whereby the pH values of probiotic cashew apple reduced from 4.28 to 3.79 after 42 days of refrigerated storage. Guo *et al.* (2009) reported that the *L. casei* reduced the pH of fermented milk from 5.59 to 4.60 after 28 days of chilled storage. Akalin *et al.* (2004) also reported a similar trend for yoghurts whereby the pH decreased from 4.51 to 4.40 after 28 days of refrigerated storage at 4°C. The probiotic strain used and whether the products were based on milk or water influence the pH levels of the probiotic products. Water-based products obtained significantly lower pH levels and faster

reduction in pH during storage (Ranadheera *et al.*, 2010). Machado *et al.* (2017) reported that the pH of the yoghurt added with different concentration of stingless bee honey continuously decreased until the 28th day of storage. The pH of yoghurt samples containing 5%, 10%, 15% of honey was decreased from 4.57 to 4.31, 4.51 to 4.27, and 4.48 to 4.21, respectively.

3.1.5 Colour analysis

Colour is the most important product-intrinsic sensory cue when it comes to setting consumers' expectations regarding the likely taste and flavour of food and drink. Colour deterioration can be caused by changes in physical, chemical or microbiological parameters in food (Coggins *et al.*, 2010). Chroma meter determines the colour of samples based on L*a*b* colour system whereby L* value represents lightness (L* = 0 indicates black while L* = 100 indicates white), a* value represents redness (positive a) and greenness (negative a) while b* represents yellowness (positive b) and blueness (negative b). Table 1 shows the effect of incorporation of honey and LAB on lightness (L*), red/greenness (a*) and yellow/blue (b*) of MMT juice within 28 days of chilled storage. One-way ANOVA test showed that there was no significant difference in the lightness of all juice samples on 1st and 7th day ($p > 0.05$). After 14th day, it is noted that there was a significant difference between the lightness of control juice and both probiotic MMT juice samples ($p < 0.05$). The lightness of both probiotic MMT juices was lower than that of the control MMT juice probably due to higher turbidity caused by the biomass given by *L. plantarum*. A similar finding has been reported in probiotic cashew apple juice as reported by Pereira *et al.* (2011).

There was no significant difference in the a* values (redness) of control and probiotic MMT juice with and without honey ($p > 0.05$). Whereas the b* values of the

Table 1. Effect of incorporation of honey and LAB on lightness (L*), red/greenness (a*) and yellow/blue (b*) of MMT juice within 28 days of chilled storage

Storage days	Samples								
	Control			Probiotic MMT juice			Probiotic MMT juice with honey		
	L* value	a* value	b* value	L* value	a* value	b* value	L* value	a* value	b* value
1	11.23±0.90 ^{Ad}	1.13±0.00 ^{Aa}	5.31±0.28 ^{Ab}	7.23±2.26 ^{Ab}	1.31±0.06 ^{Aa}	3.98±1.44 ^{Ab}	9.37±0.64 ^{Ad}	1.21±0.11 ^{Aa}	5.22±0.35 ^{Ab}
7	15.32±1.12 ^{Ac}	0.90±0.00 ^{Ab}	5.90±0.48 ^{Ab}	15.18±0.68 ^{Aa}	0.96±0.03 ^{Aab}	4.71±0.11 ^{Bab}	14.66±0.16 ^{Ac}	0.94±0.01 ^{Ab}	5.07±0.10 ^{ABb}
14	20.90±0.11 ^{Aa}	0.58±0.03 ^{Ad}	6.57±0.63 ^{Aab}	16.60±0.61 ^{Ba}	0.75±0.28 ^{Ab}	5.53±0.05 ^{Aab}	20.37±0.69 ^{Aa}	0.40±0.16 ^{Ac}	6.16±0.29 ^{Aa}
21	18.48±0.25 ^{Ab}	0.71±0.11 ^{Ac}	7.15±0.29 ^{Aa}	15.28±0.86 ^{Ba}	0.64±0.05 ^{Ab}	5.77±0.37 ^{Bab}	16.80±0.17 ^{ABb}	0.53±0.00 ^{Ac}	6.39±0.06 ^{ABa}
28	19.04±0.29 ^{Aab}	0.54±0.04 ^{Ad}	7.23±0.24 ^{Aa}	17.2±0.00 ^{Ba}	0.46±0.00 ^{Ab}	5.77±0.00 ^{Ba}	17.26±0.29 ^{Bb}	0.47±0.03 ^{Ac}	5.87±0.08 ^{Ba}

Values are expressed as mean±standard deviation. Values with different uppercase superscript within the same row are significantly different ($p < 0.05$) while values with different lowercase superscripts within the same column are significantly different ($p < 0.05$).

control MMT juice was highest compared to both probiotic MMT juices. This could be due to degradation of carotenoids by LAB or fermentation conditions such as temperature and pH (Panda *et al.*, 2007). The colour component b^* of the probiotic MMT juice with honey was higher than without honey. This may be due to the addition of the honey causing increase in yellowness of the MMT juice.

3.1.6 Titratable acidity

Figure 5 shows the effect of incorporation of honey and LAB on titratable acidity of MMT juice within 28 days of chilled storage. There was a significant difference ($p < 0.05$) in titratable acidity between control MMT juice and both probiotic MMT juices. Both probiotic MMT juices gave higher titratable acidity than control MMT juice. According to Pereira *et al.* (2012), the titratable acidity of the probiotic fruit juice increases due to the conversion of sugar into lactic acid by LAB. The titratable acidity of probiotic MMT juice with honey was higher than that without honey because it contained extra sugar from the addition of honey, leading to more sugar can be converted by LAB to lactic acid. Moreover, higher acidity in probiotic MMT juice containing honey may also be associated with the presence of prebiotic oligosaccharides in stingless bee honey because these compounds at such a small amount may promote the growth and/or the metabolic activity of LAB.

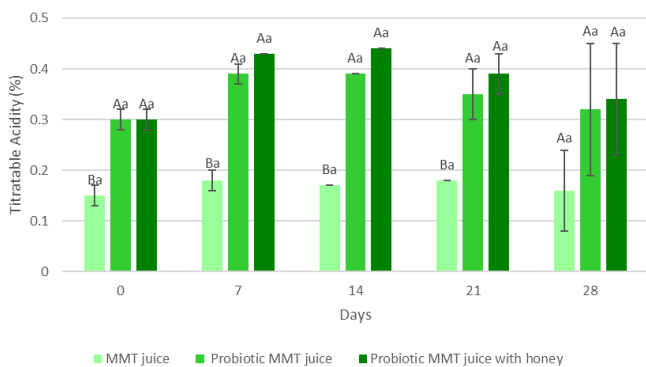


Figure 5. Effect of incorporation of honey and LAB on titratable acidity of MMT juice within 28 days of storage. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

Throughout the 28 days of chilled storage period, all samples showed no significant changes in titratable acidity. This is expected because LAB count decreased with storage time, thus there was no increase in lactic acid with storage time. Contrasting results were reported by Nualkaekul *et al.* (2001) whereby lactic acid levels increased in orange, grapefruit, blackcurrant, and

pineapple juices fermented with *Bifidobacterium longum* NCIMB 8809. Mahmood *et al.* (2019) also reported that the titratable acidity of probiotic yoghurt prepared with Dahi micro-flora improved steadily during the 28 days of storage.

3.1.7 Viscosity

Viscosity is an important characteristic to determine in the food industry because it is a good indicator of density and related to the appearance of the product (Thakur, 2017). Figure 6 shows the effect of incorporation of honey and LAB on the viscosity of MMT juice within 28 days of storage. On a similar storage day, there was no significant difference ($p > 0.05$) in viscosity in all juice samples within 28 days storage except on the 7th day. On the 7th day, the viscosity of probiotic MMT juice without honey showed the lowest viscosity (3.15 cP). This can be explained the utilization of sugar in MMT juice by probiotic microorganisms caused the decreased in the viscosity. According to Gangwar *et al.* (2018), the viscosity of the food can be influenced by the sugar content. An increase in the soluble solids can enhance the flow resistance that leads to an increase in viscosity of the liquid. Honey contains high sugar content which can increase the viscosity of the probiotic MMT juice and this caused its viscosity higher than probiotic MMT juice without honey.

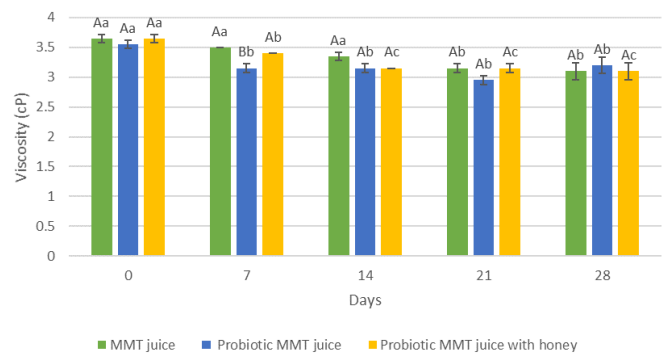


Figure 6: Effect of incorporation of honey and LAB on viscosity of MMT juice within 28 days of storage. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

Throughout 28 days of chilled storage, there was a significant decrease in viscosity after day-21 for control MMT juice ($p < 0.05$). For probiotic MMT juice with and without honey, there was a significant decrease ($p < 0.05$) in viscosity after day-7. The loss of viscosity in control MMT juice may be related to the depolymerization of pectin due to endogenous pectinase action (pectin methylesterase (PME) and polygalacturonase (PG) during storage (Aguiló-Aguayo *et al.*, 2010). Besides that, the viscosity of MMT probiotic juice with and

without honey also decreased during 28 days of storage due to the utilization of sugar by LAB. Similar finding has been reported by Mahmood *et al.* (2019) and Donkor *et al.* (2007) for probiotic yoghurt. Aryana and McGrew (2007) findings also observed a gradual reduction in the apparent viscosity of probiotic yoghurt possibly due to the activity of bacteria enzymes on the matrix of casein micelles over time.

A different finding was reported by Da Costa *et al.* (2017) who reported that there was no difference in the viscosity of the probiotic orange juice within 28 days chilled storage. The viscosity of juice also may be influenced by types and concentration of culture bacteria (Aryana and McGrew, 2007).

3.1.8 Total soluble solids (TSS)

Figure 7 shows the effect of incorporation of honey and LAB on TSS of MMT juice within 28 days of storage. One-way ANOVA test showed that there was a significant difference ($p < 0.05$) between the TSS of control MMT juice, probiotic MMT juice with and without honey on day 0. The initial TSS of probiotic MMT juice with honey was the highest which was 7.55°Brix compared to control MMT juice and probiotic MMT juice without honey which was 3.6°Brix and 4.85°Brix respectively. An increase in TSS content of the probiotic MMT juice was due to the increase in viable cell counts after fermentation (Gangwar *et al.*, 2018). With the increasing storage period, the TSS of control MMT juice increased. The increase is probably due to the hydrolysis of polysaccharides like starch, cellulose, pectin, etc. and conversion into simple sugars (Mgaya-Kilima *et al.*, 2014). On day 7 and 14, control MMT juice gave lower TSS compared to both probiotic juices. Whereas on day 21 and 28, there was no significant difference in TSS of all MMT juice samples.

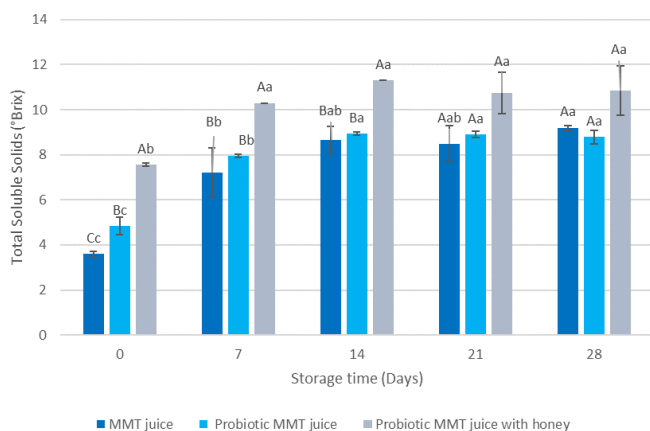


Figure 7. Effect of incorporation of honey and LAB on total soluble solid of MMT juice within 28 days of storage. Bars with different uppercase letters indicate significant difference ($p < 0.05$) between sample within the same storage day while bars with different lowercase letters indicate significant difference ($p < 0.05$) between storage days within same sample.

One-way ANOVA test showed that there was a significant difference in TSS for all MMT juice samples with increasing storage period ($p < 0.05$). The TSS of control MMT juice and probiotic MMT juice increased and kept almost constant after the 14th day until the end of storage period. For the probiotic MMT juice with honey, TSS increased from day-0 to day-7 and then maintained its TSS until the end of storage period. The action of enzymes that catalyse the bee enzyme transfer D-glucopyranosyl sucrose to a carbohydrate receptor caused the increase in sugar content in honey samples (Machado *et al.*, 2017). In addition, greater amounts of reducing sugars were released to probiotic MMT juice due to certain microbial enzymes action toward honey sugars (Machado *et al.*, 2017). Additional free sugar molecules from honey in this matrix may release due to the action of enzymes produced by LAB. These new available sugar molecules may increase the total sugars content.

Similar findings were reported by Nath *et al.* (2015) for honey probiotic beverage, whereby TSS increased after 15 days of chilled storage (24°Brix to 31°Brix). Machado *et al.* (2017) also reported that the increase in total sugar content was observed at the end of the assessed storage period for all yoghurt formulations, but this increase was more evident in formulations yoghurt with 10% and 15% of honey concentration on the 14th or 21st day of storage. However, a reduction of TSS in probiotic pineapple juice samples during storage was observed by Tayo and Akpeji (2016). They reported that the reduction of TSS was due to the metabolic activity of the probiotic LAB. Kumar *et al.* (2013) also reported a reduction of TSS in fruit juice with *L. casei*.

3.2 Sensory acceptance

The sensory evaluation was conducted by 40 untrained panellists. Sensory attributes such as colour, odour, MMT flavour, sweetness, sourness and overall acceptability were evaluated in control MMT juice, probiotic MMT juice with and without honey. Table 2 shows that the sensory acceptance of MMT juice, probiotic MMT juice with and without honey for various attributes.

There was no significant difference ($p < 0.05$) in all three formulations MMT juice samples for sourness, overall acceptability and odour attributes. This indicates that all three juice samples have similar acceptability for these three attributes. For other attributes, there was a significant difference between the three MMT juice samples. For colour attribute, control MMT juice gave a higher score compared to probiotic MMT juice with honey ($p < 0.05$). However, the colour acceptability of probiotic MMT juice without honey was not

Table 2. Sensory acceptance of control MMT juice, probiotic MMT juice without honey and probiotic MMT juice with 3.9% honey

Sample	Colour	Odour	MMT flavour	Sweetness	Sourness	Overall Acceptability
Control MMT juice	6.3±0.91 ^a	5.2±1.45 ^a	5.58±1.57 ^a	5.23±1.64 ^a	4.95±1.6 ^a	5.1±1.77 ^a
Probiotic MMT juice without honey	5.95±0.78 ^{ab}	4.58±1.55 ^a	4.58±1.58 ^b	4.33±1.4 ^b	4.2±1.74 ^a	4.65±1.56 ^a
Probiotic MMT juice with 3.9% honey	5.69±0.95 ^b	4.31±1.32 ^a	4.28±1.56 ^b	4.3 ^b ±1.7 ^b	4.18±1.78 ^a	4.39±1.53 ^a

Values are expressed as mean±standard deviation. Values with different superscripts within the same column are significantly different ($p < 0.05$).

significantly different from other samples. This result is associated with the colour analysis which means higher yellowness and total colour difference of MMT juice, higher the acceptance of the panel.

For MMT flavour, control MMT juice gave a higher score than other samples. According to Cruz *et al.* (2010), the production of components by the metabolism of the probiotic culture can contribute negatively to the aroma and taste of the product. Luckow and Delahunty (2004) investigated that the sensory characteristics attributed to the functional orange juices included “lactic aroma, medicinal taste, artificial, and earthy taste”. The authors reported that infrequent consumer did not reject the product although these attributes were completely unacceptable to frequent consumers of conventional orange juice. This result contrasted with the report by Machado *et al.* (2017), whereby they found that the goat yoghurt with the highest concentration of honey had the highest flavour acceptance. In this study, the result showed that the addition of probiotic microorganisms affects the sensory profile of the final product.

For the sweetness acceptance, MMT juice gave the highest mean score while probiotic MMT juice with and without honey showed no significant difference ($p > 0.05$) between each other. This may be due to utilisation of sugar by the probiotic microorganisms, causing reduction of sugar in probiotic MMT juice. It was also found that the addition of 3.9% honey in probiotic MMT juice gave no effect on the sweetness of the probiotic MMT juice.

Higher acceptability score was reported when fermented milk with *Bifidobacteria* was added with 5-10% honey due to the presence of pleasant flavour-forming compounds (Riazi and Ziar, 2012). Higher acidity observed in probiotic MMT juice with honey impact negatively on the sensory acceptance and reduce the original sweetness of the honey. This can be overcome by shortening the fermentation time of LAB to less than 24 hrs. Since the control MMT sample obtained the highest scores in most of the sensory attributes, thus, the most acceptable sample was the control MMT sample. Thus, it can be concluded that the addition of probiotic microorganisms and 3.9% honey negatively affected the sensory attributes of the MMT juice

samples.

4. Conclusion

For all MMT juice samples, 28 days of chilled storage resulted in increased TSS, decreased viscosity and no significant differences in titratable acidity. During chilled storage, pH of control MMT juice did not change, but pH of both probiotic MMT juices decreased. There was no yeast and mould growth in probiotic MMT juice with honey. At the 21 and 28 days of storage, control MMT juice gave higher yeast and mould count than probiotic MMT juice without honey. During chilled storage, there was no LAB count in control MMT juice, but the LAB count decreased for both probiotic MMT juices. During chilled storage, there was no significant change in biomass for control MMT juice, but the biomass decreased for both probiotic MMT juices. The most acceptable sensory formulation was the control MMT juice followed by probiotic MMT juice without honey and probiotic MMT juice with honey.

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