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Viability of Lactic Acid Bacteria (*L. Acidophilus*) in Probiotic Ready to Drink Juices

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ABSTRACT

Probiotics are consumed in dairy based goods, however, given the rise in various diet types, non-dairy alternatives, such as inoculating fruit juices with probiotics were studied for substitute. *Lactobacillus acidophilus* is a probiotic strain exerting a number of human health benefits. Therefore, the objective of this study was to determine the viability of *Lactobacillus acidophilus* in different non-dairy substrate to produce a Probiotic Ready to drink (RTD) juice blend made of malunggay, pineapple and mango over 24 hours of fermentation and 21 days of refrigerated storage. The secondary objective was to determine the sensory parameters using 9-point Hedonic scale and physicochemical properties of probiotic RTD Juice Blend in terms of pH, TSS and total acidity. All of the probiotic RTD Juice Blend samples achieved a mean viable count of at least 10^6 CFU/mL during 24 hours of fermentation and 21 days of refrigerated storage. According to the sensory evaluation, which evaluated samples according to color, aroma, taste and overall acceptability, probiotic RTD juice blend with malunggay-pineapple-mango blend proved to have the highest score for all characteristics with pH of 4.62, TSS of 13.47^{O}Bx and total acidity of 0.47%. The pH and TSS decreases with time while acidity increases. This condition is good for the production of microbes because low pH can decrease the microbes' production rate. This environment may enable LAB to successfully pass the pH of the stomach and bind in the gut of the host which is needed for successful colonization and propagation for expression of its health-promoting effects. Therefore, this study indicated a potential for probiotic fruit juices as a valid alternative to dairy based probiotic products.

Keywords: Lactobacillus acidophilus, fruit and vegetables, ready-to-drink, probiotics

1 Introduction

The development of probiotic foods is increasing day by day due to the consumer consciousness and recognition about functional foods. The importance now has moved from medication to prevention in light of the swelling cost of health care, the steady upsurge in life expectancy, and the yearning of elderly people for improved life quality.

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Probiotics are live feed supplements that enhance the health of consumers by improving the balance of micro flora in the gut when ingested live and in sufficient numbers [1]. These probiotics are claimed to deliver a number of health benefits such as including antimicrobial effects, anti-tumor, anti-cholesterol, anti-diabetic and treatment of diarrhea and lactose intolerance [2].

Yet, there are many members of society who remain barren of probiotics. The consumers' call for non-dairy based probiotic products has increased due to problems of lactose intolerance and cholesterol content associated with the dairy products. In this respect, fruits and vegetable bids a substitute for the production of probiotic foods using lactobacillus species. The nutritive content of fruit juices could prove to be an ideal candidate for the delivery of probiotics to the large segment of the population that cannot or does not want to have milk goods in their diets [3].

Malunggay was used in addressing malnutrition in emerging areas of the world. It has been used effectively to fight nutrient deficiency among infants and women of childbearing age. Malunggay contains a substantial source of vitamins and mineral while probiotic microorganisms offer beneficial effects to human health [4].

Pineapple is an economically significant plant and the third most important fruit crop in the tropical and subtropical regions of the world. Pineapple (Ananas comosus) is a tropical and economically significant plant from the family Bromeliaceae. Since pineapple juice already contains beneficial nutrients, it may serve as an ideal food matrix for carrying probiotic bacteria [5, 6]. Furthermore, it has a very pleasing taste profile to all age groups and is perceived as being healthy and refreshing.

Mango (Mangifera indica) is one of the most popular fruits in the world due to its unique flavor and excellent nutritional value. Mango contains a variety of phytochemicals and also contains nutrients [5, 7]. It is an excellent source of vitamin A, vitamin E and Selenium which help to protect against heart disease.

Although there are many studies gauging the effectiveness of probiotics in fruit juices, none have used L. acidophilus as a strain of choice in blend of malunggay, pineapple and mango. Therefore, the aim of this study is to evaluate the probiotic potential of L. acidophilus in malunggay, pineapple and mango over 21 days of storage and to determine physicochemical properties, consumer acceptability and sensory properties of the product.

2 Materials and Methods

2.1 Experimental Design

The samples was prepared in three batches to serve as replications to determine the viability of lactobacillus in the probiotic RTD Juice Blend. This experiment used Completely Randomized Design (CRD). The following treatments were used in the study:

T0 – 20% malunggay extract, 40% pineapple extract, 40% mango extract, no LAB
T1 – 20% malunggay extract, 80% pineapple extract with LAB
T2 – 20% malunggay extract, 80% mango, with LAB
T3 – 20% malunggay extract, 40% pineapple extract, 40% mango extract, with LAB

2.2 Procurement of Raw Materials

The raw materials such as malunggay leaves, pineapple and mango were bought from local market. Lactic Acid Bacteria in capsule form was bought in a pharmaceutical store. The evaluation of the viability of lactobacillus was prepared using procedure of Barcelon et al., [8] with modification of the substrate to be used for LAB. The Analytical Profile Index (API) Kit was used to verify the identities of test isolates.

2.3 Fruit Juice Preparation, incorporation of LAB and fermentation

Fresh moringa leaves, pineapple, and mango were juiced by extractor. The extracted juice were pasteurized at 80^{o}C for 5 minutes. The juice were categorized into the following treatments: i) T0 : Control; ii) T1: 20% moringa: 80% pineapple juice blend with LAB; iv) T2: 20% moringa: 80% mango juice blend with LAB; T3: 20% malunggay: 40% pineapple: 40% mango juice blend with LAB. The inoculated samples along with the control were incubated at 37^{O}C for 24 hrs [9].

2.4 Physicochemical Analysis and viability of LAB

Physicochemical properties such as pH, total soluble solids [11] and total acidity [10] (expressed as percent lactic acid) were evaluated at day 0, 7, 14 and 21. Viable cell counts in log (CFU/ml) was determined by standard plate count method using ROGOSA agar. The viability of LAB was also determined during storage of 4^{o}C at day 0, 1, 7, 14 and 21.

2.5 Sensory Evaluation

Sensory parameters such as color, aroma, taste and overall acceptability were evaluated by 20 semi-trained panelist using 9-point hedonic scale ranging from 1 for “Dislike Extremely” to 9 for “Like Extremely” [12].

2.6 Statistical Analysis

The results of sensory evaluation and physicochemical analysis was tabulated and analyzed using Friedman Test, Tukey’s Test and Analysis of Variance. Friedman test was used to determine the significant difference of the sensory parameters. The bacterial counts will be expressed as mean. ANOVA will be used to determine the significant difference of LAB counts from Day 0 to Day 21.

3 Results and Discussion

3.1 Physicochemical Properties

pH

From table 1, the pH value of different probiotic RTD juice samples decreased along with time. The pH ranges from 4.10 – 4.68. Highest pH (4.68) was found in probiotic RTD juice with malunggay-mango blend (T2) at Day 0 and lowest value (4.10) was obtained in malunggay-pineapple-mango blend (T3) for Day 21. The pH of the probiotic samples is slightly acidic. The results showed no significant change in Day 0 but shows notable significant change in its pH at Day 7, 14 and 21. The pH values of the different Juice Blends slightly reduced due to the fact that the fermentation process was still in its early stage and no lactic acid had accumulated yet [13]. From day 7 to 21, the pH values varied significantly between samples. As the days progressed, the lactic acid produced by probiotics accumulates, altering the acidity and reducing the pH of the probiotic RTD Juice Blend making it more acidic compared to the previous pH measurement. The blends of the probiotic juices shows a continuous decrease in pH signifying an ongoing conversion of sugar and other components in the drink. The result of the study coincides with the study of Viander et al. [14] claiming that the decrease in pH attributed to the sugar consumption and production of organic acid by lactic acid cultures.

Table 1. *pH of the Probiotic RTD Juice Blend*

Probiotic RTD Juice Blend	pH			
	Day 0^{ns}	Day 7*	Day 14**	Day 21**
20%malunggay-40%pineapple-40%mango, w/o LAB (T0)	4.63	4.59^{a}	4.52^{a}	4.39^{a}
20%malunggay-80%pineapple w/ LAB (T1)	4.57	4.37^{b}	4.30^{b}	4.17^{b}
20%malunggay -80%mango w/ LAB (T2)	4.68	4.53^{ab}	4.49^{a}	4.37^{a}
20%malunggay-40%pineapple-40%mango w/ LAB, (T3)	4.64	4.44^{ab}	4.23^{b}	4.10^{b}

*Means followed by a common letter are not significant at 5% Tukey’s Test
Legend: (ns) Not Significant (*) Significant (**) Highly Significant

Total Soluble Solids (TSS)

TSS content among the sample is significantly affected as storage time progresses. As the retention time increases the value of TSS decreased. The incorporation of probiotics in RTD Juice shows an impact on the TSS thereby resulting in the reduction in sugar content or other soluble components. The reasons behind that are assumed to be the breakdown of solid matters and utilization of these materials by lactic acid bacteria during storage days. The probiotics may have been actively consuming and metabolizing the soluble solids contained in the RTD Juice blends, as indicated by the declining trend in TSS. It is assumed also that, lactobacillus bacteria consumed sugar for their cell synthesis during fermentation. Kumar et al. [15] studied with mango, grape, cantaloupe and sapota and this study also found similar results.

In comparison to Mongkontanawat's [17] study, which was titled "*Fermentation of Gac juice mixture by probiotic lactic acid bacteria*," it was found that the amount of total soluble solids in the fermented Gac juice decreased significantly as the fermentation time increased, with the lowest level occurring at 72 hours of fermentation, or 8.6°Brix. These results were the same as the findings of this study in the total soluble solids of the probiotic RTD juice blend, in which as the fermentation progresses, there is a decrease in the TSS of the drink.

Probiotic consumption of soluble solids is crucial to the fermentation process as it helps the fermented beverage produce the required qualities, flavor and taste which is improved through fermentation time.

Table 2. *TSS of the Probiotic RTD juice*

Probiotic RTD Juice Blend	Total Soluble Solids (°Bx)			
	Day 0**	Day 7**	Day 14**	Day 21**
20%malunggay-40%pineapple-40%mango, w/o LAB (T0)	13.49[a]	12.14[a]	11.13[a]	10.62[a]
20%malunggay-80%pineapple w/ LAB (T1)	11.52[b]	10.81[c]	7.68[d]	7.29[c]
20%malunggay -80%mango w/ LAB (T2)	10.55[b]	10.55[d]	7.89[c]	7.51[b]
20%malunggay-40%pineapple-40%mango w/ LAB, (T3)	13.31[a]	12.46[b]	8.42[b]	7.43[b]

*Means followed by a common letter are not significant at 5% Tukey's Test

Legend: (ns) Not Significant (*) Significant (**) Highly Significant

Total Acidity(%TA)

Acidity has a relation with pH and it is vice versa. RTD Juice blends with LAB showed an increase in TA as the days progressed. With the reduction in the pH, the acidity content increased. The lactic acid increased the acidity content. Yoon et al. [16], and Sharma and Mishra [18] studies also agreed the increase rate in acidity content with time. The total acid content of the probiotic RTD Juice Blend increased with the time. As the time increases the population of the bacteria also increase and they produce more lactic acid thereby reducing the pH and TSS of the probiotic drink. The findings of acidity also increased significantly after day 1 to day 7 which is the same as the study of Sharma and Mishra [18] that acidity increased significantly till 72 hours and then starts reduction. . The increased titratable acidity in probiotic beverages is due to the accumulation of lactic acid during the prolonged fermentation process. The study found that as fermentation time increased, lactic acid production increased, resulting in a higher total acidity in the fermented drink. Relative differences concerning the lactic acid increasing were observed in all treatments.

Table 3. *Total Acidity of the Probiotic RTD juice*

Probiotic RTD Juice Blend	Total Acidity (%)			
	Day 0**	Day 7**	Day 14**	Day 21**
20%malunggay-40%pineapple-40%mango, w/o LAB (T0)	0.43[b]	0.56[c]	0.79[bc]	0.74[c]
20%malunggay-80%pineapple w/ LAB (T1)	0.56[a]	0.73[ab]	0.84[b]	1.19[b]
20%malunggay -80%mango w/ LAB (T2)	0.28[c]	0.68[b]	0.72[c]	0.84[c]
20%malunggay-40%pineapple-40%mango w/ LAB, (T3)	0.49[ab]	0.81[a]	0.94[a]	1.46[a]

*Means followed by a common letter are not significant at 5% Tukey's Test
Legend: (ns) Not Significant; (*) Significant; (**) Highly Significant

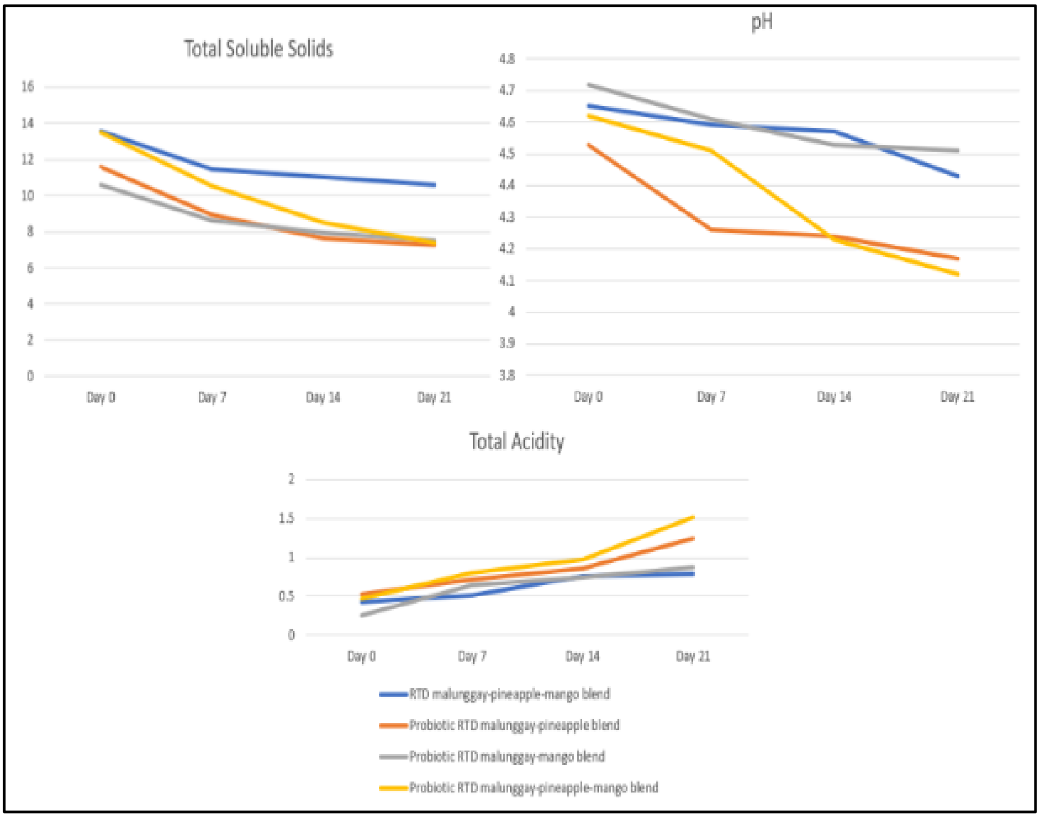

Figure 1. *Changes in Physicochemical properties of Probiotic RTD Juice Blend with Time*

3.2 Viability of LAB

Microbial growth behavior in the probiotic RTD Juice Blend was also studied as microbial growth stability is one of the most important parameter for probiotic juice. Initially the microbial count was increased with time due to population increase but in Treatment 0 – control it was null because no microbial strains were used in that sample. For samples with probiotics, there was an increase in the count from Day 1 (7.70 log CFU/ml) to Day 7 (8.92 log CFU/ml) which signifies the Log phase of the growth curve of the probiotics. After day 7 (8.92 log CFU/ml), microbial count started to decrease. The same on the study of Hossain et al., [19] with noticeable decrease from the initial count after Day 7 and then slowly starting to decline at Day 14 and Day 21. The blend of malunggay-pineapple-mango showed best performance in terms of population density than other treatment with malunggay-pineapple and malunggay-mango blend until day 21. It has been observed that the viability of the *L. acidophilus* was excellent, meaning almost all viable cells were retained after 21 days storage The viable cell population for Day 7 and after Day 21 is within the standard

(≥1.0x10⁶ cfu ml-1). The results indicated that, *Lactobacillus acidophilus* were able to survive and utilized malunggay, pineapple and mango blend juice for their cell synthesis, which was indicated by a decrease in sugar and pH, and increase in acidity. It was also observed that, *Lactobacillus acidophilus* were able to withstand and increase in number during 24 hours of fermentation.

Table 5. *Viability of Lactobacillus acidophilus in Probiotic RTD Juice*

Probiotic RTD Juice Blend	Number of log (cfu/mL) of Probiotic RTD Juice				
	Day 0	Day 1	Day 7	Day 14	Day 21
20%malunggay-40%pineapple-40%mango, w/o LAB (T0)	0	0	0	0	0
20%malunggay-80%pineapple w/ LAB (T1)	7.70	8.85	8.95	7.72	7.62
20%malunggay -80%mango w/ LAB (T2)	7.70	8.99	8.92	7.80	6.94
20%malunggay-40%pineapple-40%mango w/ LAB, (T3)	7.70	8.94	8.97	8.80	7.97

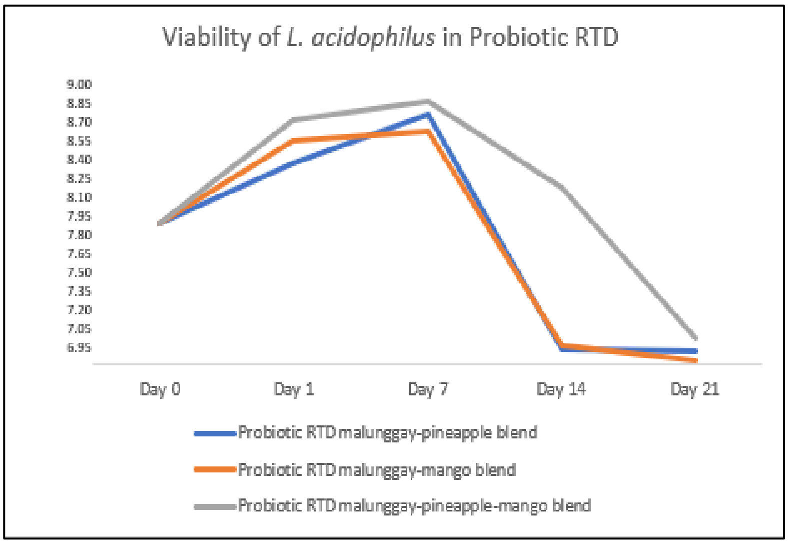

Figure 2. *Viability (log CFU/ml) of Probiotic RTD Juice Blend with Time*

3.3 Sensory Properties

From the result of sensory evaluation, it was found that the probiotic juice has almost the same kind of color and aroma level as fresh juice. The taste and overall acceptability showed significant difference and was affected by the type of blend / substrate used in probiotic juice. The profile of the probiotic juice with malunggay-pineapple-mango blend was almost similar to the control but in most cases people like probiotic juice more than fresh juice due to the promising health benefits it could convey. The results are shown in table 5.

Color. The result showed that the treatments with different blend of malunggay, pineapple and mango probiotic RTD juices are not significantly different which means that the appearance or color of the product is comparable to the control thus changing the substrate of the probiotic RTD juice blend does not affect its color. The color is dark green to army green for all of the treatment even different proportion of fruits were used. The green color of malunggay was brought about by the pigment chlorophyll found in the leaves of green plants that contributed to the color of the probiotic RTD juice blend.

Aroma. The aroma of the samples are not significant thus all treatments are comparable to the control in terms of its aroma and different substrates for probiotic RTD juice blend does not impart change in aroma. It is perceived that the sample still smelled more of moringa even though large portion of the blends were fruits. Aroma attribute was detected by the nose through the volatile compounds the food contains and emit. The odor helps the consumers recognize dangers as off-odor can indicate that the food may be spoiled

[20]. It is observed that the mean aroma acceptability ranges from 7.30-7.60 which is regarded by panelist as moderately desirable. These findings were the same to study of Dhillon et al., [21] which found that aroma of mango juice was considered to be a major characteristic of fermented drinks. It infers that probiotic inoculation has an impact on the volatile component synthesis of fermented drinks, which in turn changes the overall aroma.

Taste. The taste of the probiotic juices was evaluated the highest with malunggay-pineapple-mango blend and was rated as Like Very Much. Chemical components of malunggay have volatile and non-volatile substances responsible for the basic taste of bitterness, and on the other hand, pineapple and mango has its significant components that affect the sweetness such as D-glucose, D-fructose, and sucrose while for sourness, it composed of substances such as, L-malic acid and citric acid [22].

Overall Acceptability. It is observed that upon evaluating all the treatments in terms of its organoleptic properties, probiotic RTD Juice Blend with malunggay-pineapple-mango blend was the most acceptable formulation (8.21) that fall under the Like Very much rating and is significantly different to other treatments. Sensory attributes or organoleptic properties are important factors that determine consumer's acceptability of new product.

Table 6. *Mean Score for sensory evaluation of different blend of Probiotic RTD Juice*

Probiotic RTD Juice Blend	Sensory Parameters			
	Color^{ns}	**Aroma^{ns}**	**Taste****	**Overall Acceptability****
20%malunggay-40%pineapple-40%mango, w/o LAB (T0)	**8.15**	**7.50**	**8.29^{a}**	**7.82^{b}**
20%malunggay-80%pineapple w/ LAB (T1)	**8.24**	**7.60**	**7.53^{c}**	**7.60^{b}**
20%malunggay -80%mango w/ LAB (T2)	**8.32**	**7.30**	**7.91^{b}**	**7.39^{c}**
20%malunggay-40%pineapple-40%mango w/ LAB, (T3)	**8.30**	**7.60**	**8.41^{a}**	**8.21^{a}**

Note: **Means followed by different letters are significantly different at 5%probability level of Tukey's tests. Legend: **: Highly significant, *: Significant; ^{ns}: Not significant
Color: 9; extremely desirable to 1; extremely undesirable
Aroma: 9; extremely desirable to 1; extremely undesirable
Taste: 9; Like extremely to 1; Dislike extremely
General Acceptability: 9; extremely acceptable to 1; extremely unacceptable

4 Conclusion and Recommendation

The results of the study demonstrated that *Lactobacillus acidophilus* were able to survive in fermented juice blends of malunggay, pineapple and mango with high acidity and low pH. Among four different treatments, probiotic RTD juice blends of malunggay, pineapple and mango which has been made by fermentation with *L. acidophilus* exhibited the best results in terms of physicochemical, microbial and sensory characteristics. Therefore, it could be suggested that blends of malunggay, pineapple and mango juices would be exploited as a carrier/medium for the fermentation and delivery of probiotic lactic acid bacteria, and these probiotic-fortified products could be used as a functional healthy beverage to promote better health and nutrition of the population, especially for the milk allergic or lactose intolerant people. However, more extensive experiments are necessary in order to authenticate the probiotic potential and safety of such cultures and fruit products based on these beneficial microbes before being endorsed for the better health and nutrition of society. Thus, it is recommended to further study encapsulation of probiotic strain that could be considered for future research to determine if it would increase the viability and sensory characteristics of the probiotic juices. Furthermore, future in vivo studies on the efficacy of the probiotic juice be performed to further establish that blends of malunggay-pineapple-mango is an ideal substrate for probiotic microorganisms.

5 Declaration

5.1 Acknowledgments

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5.2 Competing Interests

The authors declared that they do not have any known conflict of interest in publishing this work.

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