FERMENTATION OF PINEAPPLE, MANGO AND BANANA JUICE USING *LACTOBACILLUS ACIDOPHILUS* 150

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ABSTRACT

Fruit juices have been considered great alternative to milk in some traditional probiotic dairy products as they contain numerous nutrients such vitamins, minerals, phytochemical compounds, etc. Therefore, the aim of this study was to evaluate the suitability of some tropical fruit juices for the growth of probiotic bacteria. *Lactobacillus acidophilus* 150 was used to ferment pineapple (*Ananas comosus L.*), mango (*Mangifera indica L.*) and banana (*Musacea musa*) juice at 37 °C/16 h. After fermentation, the highest cell number was recorded in the case of mango juice with 8.5 log CFU/mL. The pH of the investigated juices was obtained in a range of 4.07-4.32. The sugars (glucose and fructose) in juices were utilized by *L. acidophilus* 150. The highest sugar utilization was observed in the case of mango juice with 2.9% (w/v) in glucose and 1.25% (w/v) in fructose. Lactic acid was produced during the fermentation of pineapple, mango, and banana juice in the quantities of 1.94, 1.4, and 1.47% (v/v), respectively. Total phenolic content and antioxidant activity of juice presented a decrease sharp during fermentation and storage. These data highlighted that the selected tropical juices can be used for the production of probiotic products using *L. acidophilus* 150.

Keywords: Probiotics, *Lactobacillus*, tropical fruit juice, fermentation, probiotic drinks

1. INTRODUCTION

Nowadays, consumers are aware of the significant correlation between diet and health status [1, 2]; thus, the increased demand for functional food is definitely observed. In order to develop nutritionally designed foods that promote health through gut microbial reactions, three different types of food ingredients can be used: living microorganisms (probiotics), non-digestible carbohydrates (dietary fiber and prebiotics), and bioactive plant secondary metabolites such as phenolic compounds [3]. Probiotics are defined as living microbial supplements, which beneficially affect the host by improving its intestinal microbial balance, preventing colon cancer, strengthening the immune system, reducing serum cholesterol level, stimulating calcium absorption, synthesis of vitamins such as vitamin B, nicotinic acid, folic acid [4, 5]. The most commonly used probiotic bacterial genera are *Lactobacillus* and *Bifidobacterium*.

Besides probiotics, the term “postbiotics” has emerged to denote metabolites and/or cell-wall components, secreted by living bacteria or released after bacterial lysis, with demonstrated beneficial activities in the host [6]. The soluble factors secreted by living bacteria including short-chain fatty acids (SCFAs), enzymes, peptides, vitamins, and organic acids might offer physiological benefits to the host by providing additional bioactivity.

Several foods are naturally abundant in probiotics and postbiotics (e.g., yoghurt, kefir, pickled vegetables and kombucha). Yoghurt and milk-based products accounted for 65% of the global probiotic drink market in 2019 [7]. Due to technological advantages and favourable taste, milk has emerged as the most suitable medium for probiotic products [8]. However, these products cannot be consumed by groups who suffer from lactose intolerance or have allergies to milk protein. According to Perricone *et al*., up to 70% of the world population is affected by lactose-intolerance [9].

Fruits contain many essential nutrients such as vitamins, minerals and antioxidants, which naturally have health-promoting effects for the human body. Therefore, they have been recommended as a suitable medium for functional health ingredients. Prado *et al.* revealed that fruit juices could serve as a great alternative carrier in some probiotic products [10].

However, the use of fruit juice may face some challenges that need to be overcome such as the survival and viability of probiotics, potential sensory problems, etc. Fruit juices with a low pH value (pH 4.5 or below) and an insufficient source of amino acid may influence the viability and activity of bacteria during fermentation and storage. Therefore, the main objective of this study was to investigate the suitability of some tropical fruit juices including banana, mango and pineapple juice for the growth of *Lactobacillus acidophilus* 150, as well as, monitor the survival of the probiotic bacterium during 4 weeks of refrigerated storage at 4 °C.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Fruit juices

Commercial pineapple, mango and banana juice (named Rauch Happy Day) were purchased from the local market. The fruit contents in the products are 100% pineapple, 23% mango and 30% banana, respectively. The juice pH was adjusted to pH 6.4 with 4 N NaOH before fermentation.

2.1.2. Microorganisms

Lactobacillus acidophilus 150 (from Exquim SA, Spain) was used in this study. The stock culture was prepared by suspending the lyophilized *Lactobacillus* strain in MRS broth. After that, the cultures were incubated for 24 h at 37 °C. Then 1 mL of the culture was transferred into 10 mL of the MRS broth and incubated at the same previous method. The viable cell count of *Lactobacillus* strain in the MRS broth after 24 hours was in range of 8.5-9.5 log CFU/mL. The culture was used as a starter to the juice fermentation.

2.1.3. Chemicals

De Man, Rogosa, and Sharpe (MRS) broth contained (per liter) proteose peptone 10 g, yeast extract 8 g, meat extract 8 g, glucose 20 g, sodium acetate 5 g, tri-ammonium citrate 2 g, K_2HPO_4 2 g, $MgSO_4$ 0.2 g, $MnSO_4$ 0.05 g and Tween80 1 mL.

Agar medium is the medium supplemented by agar in a concentration of 15 g/L.

Other chemicals: Folin-Ciocalteu reagent, tripyridyltriazine (TPTZ), Iron (II) sulfate ($FeSO_4$), gallic acid and other standards (glucose, fructose, lactic acid, and acetic acid) for HPLC analysis, as well as pepsin and bile salts, were purchased from Sigma–Aldrich.

2.2. Methods

2.2.1. Experimental design

150 mL Erlenmeyer flasks containing 50 mL juice were inoculated with 1% of individual culture from MRS, so finally, the initial cell concentration in juices was around 6.8 log CFU/mL. The inoculated samples with *Lactobacillus* strain were conducted in an incubator for 16 h at 37 °C. After incubation, the fermented fruit juices were stored at 4 ºC for four weeks. The samples were taken at intervals every 4 hours of fermentation and two weeks of storage. The stability of the products during fermentation and storage was investigated through the changes in cell number, pH value, lactic acid concentration, reducing sugar (glucose and fructose) quantity, total phenolic content, and antioxidant activity.

2.2.2. Sample analysis

Viability of probiotic strains and measurement of pH

The cell numbers of *Lactobacillus acidophilus* 150 were determined by the pour plate method [11]. In detail, 10-fold dilution series was started with transferring 0.5 mL of sample into a test tube containing 4.5 mL saline (0.85%). Once the dilution was made, 50 µL aliquot was transferred into the 60 mm dishes. Afterward, MRS agar medium was poured into the dishes. Then all the plates were incubated at 37 °C for 48 h-72 h.

The pH values were measured by using a pH meter (Mettler Toledo, Switzerland).

Organic acid and sugar quantification

Carbohydrate and organic acid content were determined by HPLC method [11]. Briefly, the fermented juices were centrifuged at 14,000 rpm for 10 min before the supernatants were separated and filtered through 0.45 µm membrane. The analysis was performed using the surveyor HPLC system (Thermo Scientific Corporation, USA). An Aminex HPX-87H ion exclusion column with refractor index (RI) and photodiode array (PDA) detectors was used to detect sugars and organic acids, respectively. 5 mM H_2SO_4 was used as the mobile phase. The temperature of the column was maintained at 45 °C, and the running time was 25 min. The data acquisition and integration were performed using the ChromQuest 5.0 software package. Results were calculated using standard curves of corresponding sugar and organic acid.

Total phenolic content

The content of total polyphenols in fermented juices was determined using Folin's phenol reagent method [12] which has some modifications. Briefly, samples from the fermented juices were centrifuged at 14,000 rpm for 10 min. 0.2 mL of properly diluted samples were mixed thoroughly with 1 mL of 10% Folin-Ciocalteu reagent. Afterward, the mixtures were incubated for 8 min at room temperature before adding 0.8 mL of 7.5% solution of Na_2CO_3. After 15 min of heating at 50 °C, the mixtures' absorbances were measured by spectrophotometer (Unicam Helios UV/Vis) at a wavelength of 765 nm. Results were calculated using a gallic acid standard curve and expressed as a mg gallic acid equivalent (GAE) per 100 g of fermented fruit juice.

Antioxidant capacity

The antioxidant activity was evaluated following ferric reducing antioxidant power (FRAP) assays [13]. Five-fold dilution of supernatant fluids (14,000 rpm for 10 min) was mixed with 1.5 mL of FRAP reagent, which was prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM tripyridyltriazine (TPTZ) prepared in 40 mM HCl, and 20 mM ferric chloride solution ($FeCl_3$) with a ratio of 10:1:1. The mixtures were maintained at 37 °C in a water bath for 10 mins, and the absorbances were measured at 593 nm. The results were compared to a $FeSO_4$ calibration curve and expressed as a ferrous equivalent per 100 g of fermented fruit juice.

2.2.3. Statistical analysis

Results obtained were presented as mean ± SD. Values were performed from the average of triplicated experiments. One-way ANOVA was used to evaluate the statistically significant difference between the variables.

3. RESULT AND DISCUSSION

3.1. Changes in pH value and viability

The change in pH value of the juices during fermentation and storage was presented in Fig. 1. The pH of the fermented juices decreased with the rising fermentation and storage time. In detail, after 16 h of fermentation, the pH of the juices inoculated with *Lactobacillus acidophilus* 150 dropped from the initial pH 6.4 to approx. pH 4.0 in pineapple juice, pH 4.2 in mango juice and pH 4.3 in banana juice. The pH values in fermented pineapple juice were recorded the lowest among the products.

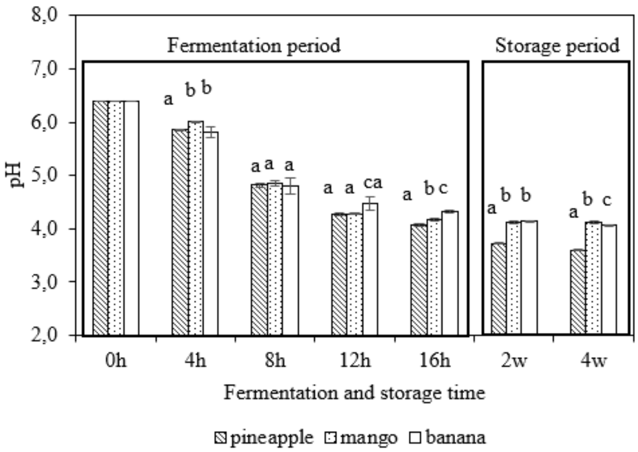

Figure 1. pH values of pineapple, mango and banana juice fermented at 37 °C for 16 h using *Lactobacillus acidophilus* 150, and further stored for 4 weeks at 4 °C. Different lowercase letters indicate the statistically significant difference ($p \leq 0.05$) between the juices for each fermentation and storage time.

During storage, the pH value of fermented juices slightly decreased in the cases of mango and banana juice. Meanwhile, the value dropped significantly in the case of pineapple juice.

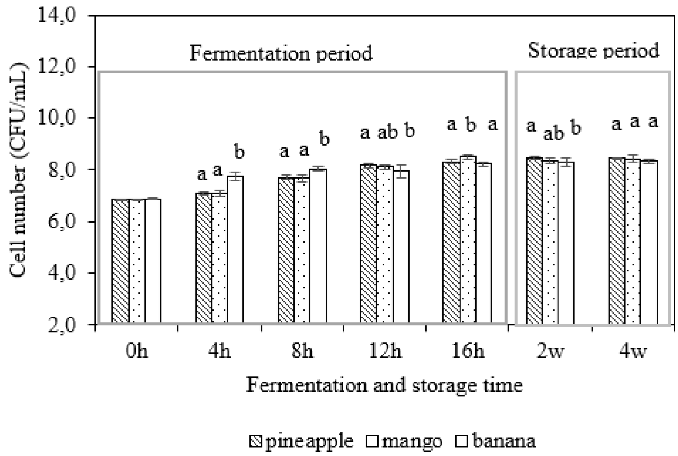

Figure 2. Cell numbers of *Lactobacillus acidophilus* 150 in pineapple, mango and banana juice during fermentation at 37 °C for 16 h, and further stored for 4 weeks at 4 °C. Different lowercase letters indicate the statistically significant difference ($p \leq 0.05$) between the juices for each fermentation and storage time.

The viable cell number of microorganisms is one of the most important criteria for probiotic products. According to Pereira *et al.*, the probiotic products should contain a significant number of probiotic bacteria over 10^7 CFU/mL for health benefits [14]. The data illustrated that *Lactobacillus acidophilus* 150 could grow well in all investigated juices without any supplementation of nutrients (Fig. 2). The microbial population of the juices after fermentation was in the range of 8.2-8.5 log CFU/mL and kept stable during four weeks of storage. Mango juice was recorded as the most suitable media for the growth of *Lactobacillus* strain because the number of this strain in the fermented juice was significantly higher than others. However, no significant statistical difference was observed between the fermented juices after four weeks of refrigerated storage.

Fermentation of pineapple, mango and banana juice using probiotic LAB as starters were also carried out by other researchers. Their results indicated that these juices supported the growth of the bacteria which is consistent with the findings in this study. Reddy *et al.* reported that *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, and *L. casei* could grow well in mango juice during fermentation at 30 °C for 72 h [15]. The final cell number exhibited in fermented juice was in a range of $1.5\text{-}2.2\times10^9$ CFU/mL. An increase viability of *L. acidophilus* from around 10^5 to 10^6 CFU/mL after fermentation of banana juice for 80 h at 37 °C was reported by Tsen *et al.* [16]. In these studies, the fermentation time is much longer compared to this study (16-24 h). This is because of the difference in the initial pH of juices. These authors used the juices without adjusting the pH value which ranged from 4.5-5.5, while in the present study, the pH was adjusted to around 6.4. Reddy *et al.* observed that the bacteria had to pass a longer lag phase (in the first 12 h) due to the stress induced from the differences between the pre-culture medium and the fermentation medium, as well as the low pH condition of the juice [15]. Reddy *et al.* also investigated the stability of fermented mango juice during 4 weeks of storage [15]. The result indicated a significant decrease in cell number of *L. plantarum* as well as of other strains after storage. They explained that the reduction in the sugar level, an accumulation of organic acid and storage temperature resulted in the reduction of probiotic viability. However, in this research, the viability of LAB strains remained stable during 4 weeks of storage at 4 °C. A significantly lower pH value (pH 3.2) of fermented juice in the study of Reddy *et al.* compared to the pH value of the fermented juice in this study may introduce a detrimental condition to the viability of probiotic bacteria during cold storage [15]. Hence, pH adjustment of juice before fermentation may offer a positive effect on the growth and survival of probiotic bacteria during fermentation and storage.

3.2. Changes in sugar and organic acid

Generally, fruit juices are a rich source of sugar. In this study, the mango juice product contains the highest sugar quantity with 7.75% (w/v) glucose and 5.45% (w/v) fructose, followed by banana with 7.61% (w/v) glucose and 4.26% (w/v) fructose. Pineapple juice was registered as the lowest sugar content with 5.9% (w/v) glucose and 3.43% (w/v) fructose.

The changes in sugar content of the juices during fermentation and storage are shown in Fig. 3. The concentration of glucose and fructose in juices decreased significantly during fermentation and storage.

After 16 hours of fermentation, sugar content in pineapple juice decreased by around 1.6% (w/v) in glucose and 0.47% (w/v) in fructose. In mango juice, the concentration dropped by 2.9% (w/v) in glucose and 1.25% (w/v) in fructose. *Lactobacillus* strain utilized around 1.3% (w/v) glucose and 0.48% (w/v) fructose in banana juice after fermentation. Glucose was observed as the preferable carbohydrate source for these strains.

The sugar utilization properties of *Lactobacillus* strains were also reported in a study of Mousavi *et al.* [17]. They revealed that the rate of glucose consumption of *Lactobacillus* strains (*L. plantarum* and *L. acidophilus*) was significantly higher than that of fructose in pomegranate juice. After fermentation, the glucose and fructose concentration of the juice inoculated with *L. plantarum* reduced by 26.8% and 16.22%, respectively. These results are very close to a report of Hashemi *et al.* [18]. The authors fermented lemon juice with *L. plantarum* LS5. After fermentation, they observed a reduction of a 26.09% glucose and 13.11% fructose in the juice. However, an opposite observation was reported by Pereira *et al.* [14]. They presented that fructose was the most consumed carbohydrate source of *L. casei* (84.76%), followed by glucose (62.1%) and sucrose (34.52%) during fermentation of cupuassu beverage. This can be explained that the metabolism of carbohydrates by probiotic bacteria varies from strain to strain and depends on the substrate and also on the fermentation conditions [17].

Organic acids, in particularly lactic acid, are accumulated during carbohydrate metabolism of LAB [19, 20]. They have an essential effect on the stability of the product by undesirable microbe restriction/inhibition [11, 14]. In comparison to other organic acid, lactic acid is registered as a major product of sugar metabolism of *Lactobacillus* during fermentation, resulted a decrease in the pH value of medium.

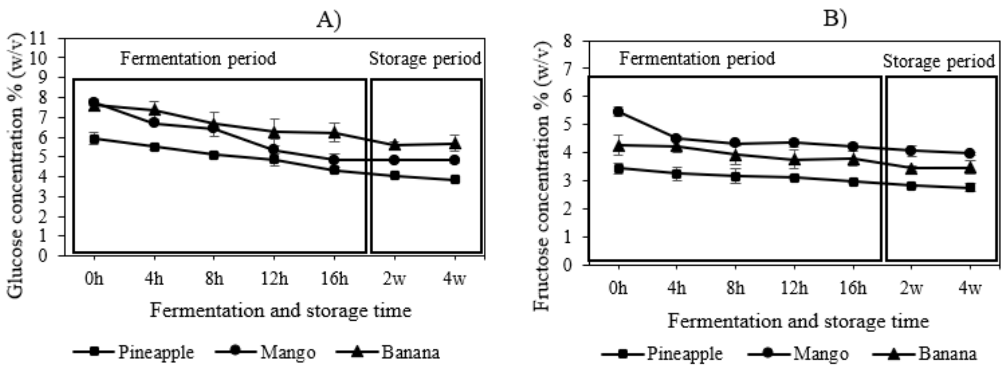

Figure 3. Glucose (A) and fructose (B) concentration in pineapple, mango and banana juice fermented at 37 °C for 16 h using *Lactobacillus acidophilus* 150, and further stored for 4 weeks at 4 °C

The change in lactic acid concentration of the juice during fermentation and storage are shown in Fig. 4. The quantity of lactic acid increased significantly during fermentation and storage. After 16 h of fermentation, the lactic acid concentration of the juices increased by 1.92% (v/v) in pineapple juice, approx. 1.4% (v/v) in mango and banana juice. Fermented pineapple juice had the highest lactic acid concentration in all investigated juice. After four weeks of storage, a significant increase in lactic acid quantity was observed. The lactic acid concentration of pineapple juice inoculated with *Lactobacillus* strain increased by 2.01% (v/v) in pineapple juice, 1.76% (v/v) in mango juice and 1.59% (v/v) in banana juice.

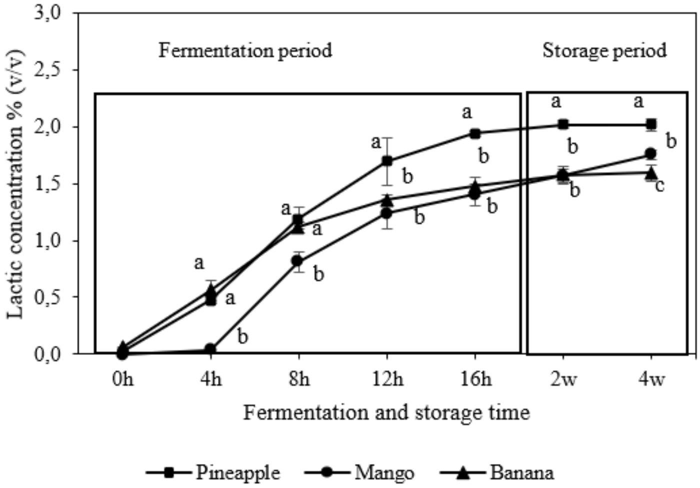

Figure 4. Lactic acid concentration of pineapple, mango and banana juice fermented at 37 °C for 16 h using *Lactobacillus acidophilus* 150, and further stored for 4 weeks at 4 °C. Different lowercase letters indicate the statistically significant difference ($p \leq 0.05$) between the juices for each fermentation and storage time.

In short, sugar metabolism of *Lactobacillus* in juices produced lactic acid as the main final product. Fermented pineapple juices had the highest value of lactic acid during fermentation and storage.

3.3. Changes in total phenolic content and antioxidant capacity

Polyphenols are beneficial compounds found in fruits, vegetables and cereals. They strongly correlate with antioxidant properties that may be involved in health improvement or pathogen restriction [21]. In the present study, total phenolic content (TPC) and antioxidant activity (FRAP) of juices were investigated during fermentation and storage. The change of TPC and FRAP values are shown in Fig. 5. Both TPC and FRAP values of fermented juices showed a slight decrease during fermentation and a significant reduction during storage. The data presented that pineapple juice had the highest TPC value which was 0.45 ± 0.01 (µg/mL gallic acid), followed by mango juice with 0.4 ± 0.01 (µg/mL gallic acid) and banana juice with 0.33 ± 0.03 (µg/mL gallic acid). The TPC content decreased by the range of 3.01-7.8% after fermentation and reached 14.75-16.78% after storage.

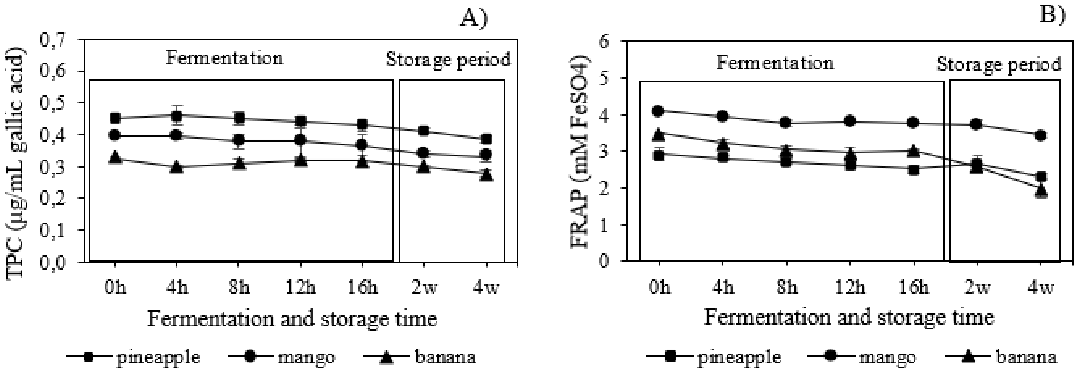

Figure 5. Total phenolic content (A) and antioxidant activity (B) of pineapple, mango and banana juice fermented at 37 °C for 16 h using *Lactobacillus acidophilus* 150, and further stored for 4 weeks at 4 °C

Regarding antioxidant activity, the highest FRAP value was observed in mango juice (4.11 mM $FeSO_4$), followed by banana (3.49 mM $FeSO_4$). The antioxidant activity of pineapple juice was the lowest with 2.93 mM $FeSO_4$. After 16 h of fermentation, the antioxidant activity of juices dropped by approx. 8.09-13.78%. After four weeks of storage at 4 °C, antioxidant capacity of fermented juice decreased in a range of 16.28-42.76%. The lowest decline in antioxidant activity was reported in the case of fermented mango juice (16.28%). A more significant decrease in FRAP was observed in pineapple (21.04%) and banana juice (42.76%) at the end of storage period. Additionally, the decrease of antioxidant activity showed that it was greatly influenced by the total phenolic content of the sample. The Pearson correlation coefficients between total phenolic content and antioxidant observations are presented in Table 1. It appeared that antioxidant capacity had a positive correlation with phenolic content. In all the investigated juices, the correlation was strong which has the r^2 value in the range of 0.852-0,885. This suggested that the phenolic content affected the antioxidant activity of the juices. Hence the changes in phenolic content could explain the changes in the antioxidant activity.

Table 1. Correlation of TPC and FRAP in fermented juices using *Lactobacillus acidophilus* 150

	Fermented pineapple juice	Fermented mango juice	Fermented banana juice
Correlation of TPC and FRAP	.853*	.885**	.852*
*. Correlation is significant at the 0.05 level (2-tailed).			
**. Correlation is significant at the 0.01 level (2-tailed).			

A similar trend in the change in TPC and FRAP of juice during lactic acid fermentation was found in a study of Tran *et al.* [22]. A reduction in phenolic content and antioxidant activity of grapefruit fermented with *L. plantarum* after fermentation was reported. The TPC and FRAP values decreased by 3.75% and 7.82%, respectively. Panda *et al.* revealed that the total phenolic content of prickly pear decreased from 0.45 µg/mL to 0.41 µg/mL after fermentation with *L. fermentum* [23]. These authors also mentioned a decrease in antioxidant activity of the lacto-juice (22.22%). Khezri *et al.* observed a significant decrease in the TPC and antioxidant activity of fig juice fermented with *L. delbrueckii* during 28 days of storage [24]. They recorded an approx. 30% and 17% reduction of these parameters, respectively. Kim *et al.* showed a decrease in antioxidant property of potato juice fermented with *L. casei* during 72 h of fermentation [25]. Jaiswal and Abu-Ghannam revealed that fermentation might affect negatively on the polyphenolic content [26]. They found an approx. 15% and 24% TPC reduction in cabbage juice fermented with *L. plantarum* and *L. rhamnosus*, respectively. A loss of 5%-13% antioxidant activity in the juice after 24 h of fermentation was also observed in their study. These authors claimed that enzymes such as β-glucosidase, p-coumaric acid decarboxylase, decarboxylase produced by LAB may be responsible for the breakdown of certain phenolic compounds.

4. CONCLUSION

In this study, all the investigated juices, particularly mango juice, were demonstrated to be good media for the growth of *Lactobacillus acidophilus* 150 without any nutrient supplementation. The *L. acidophilus* 150 could grow and maintain their viability (over 8 log CFU/mL) in the juices during fermentation and refrigerated storage. During fermentation and storage period, the probiotics utilized sugars (glucose and fructose) of the juices for its growth and produced lactic acid with high amount. Slight decreases in total phenolic content and antioxidant activity of the juices during both fermentation and storage processes were observed. This study provided promising results for the development of new non-dairy probiotic food products.

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TÓM TẮT

LÊN MEN NƯỚC CHUỐI, XOÀI VÀ DỨA SỬ DỤNG VI KHUẨN *LACTOBACILLUS ACIDOPHILUS* 150

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Nước ép trái cây được xem xét như một môi trường thay thế tuyệt vời cho sữa trong sản xuất các sản phẩm probiotic truyền thống có nguồn gốc từ sữa. Bởi vì nó chứa nhiều thành phần dinh dưỡng như vitamin, khoáng, các hoạt chất có hoạt tính sinh học… Do đó, mục đích của nghiên cứu này là để đánh giá sự phù hợp của một số loại nước ép trái cây nhiệt đới cho sự phát triển của vi khuẩn probiotic. *Lactobacillus acidophilus* 150 được sử dụng để lên men nước ép dứa (*Ananas comosus* L.), xoài (*Mangifera indica* L.) và chuối (*Musacea musa*) ở điều kiện 37 ^{o}C/16 giờ. Sau quá trình lên men, hàm lượng tế bào vi sinh vật cao nhất được ghi nhận trong nước xoài (8,5 log CFU/mL). Giá trị pH của các nước loại nước trái cây lên men nằm trong khoảng 4,07-4,32. Đường trong nước trái cây (bao gồm glucose và fructose) được sử dung bởi *L. acidophilus* 150. Lượng đường trong nước xoài được sử dụng nhiều nhất với 2,9% (w/v) đối với glucose và 1,25% (w/v) đối với fructose. Acid lactic được sản xuất trong quá trình lên men nước dứa, xoài và chuối với các giá trị lần lượt là 1,94; 1,4 và 1,47% (v/v). Các giá trị phenol tổng và khả năng chống oxy hóa của các loại nước trái cây giảm dần trong suốt quá trình lên men và bảo quản. Các kết quả này chứng tỏ rằng các loại nước trái cây nhiệt đới trong nghiên cứu này phù hợp để sử dụng cho việc sản xuất sản phẩm nước uống probiotic sử dụng *L. acidophilus* 150.

Từ khóa: Probiotics, *Lactobacillus*, nước trái cây nhiệt đới, lên men, nước uống probiotic.