HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

PRODUCTION OF PROBIOTIC TROPICAL FRUIT JUICES BY FERMENTATION WITH PROBIOTIC BACTERIA

The Theses of the PhD Dissertation

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1. INTRODUCTION AND OBJECTIVES

Nowadays, consumers are aware of the significant correlation between diet and health status; thus, the increase in the demand of functional food is definitely observed. In order to develop nutritionally designed foods that promote health through gut microbiome, three different types of food ingredients can be used: living microorganisms (probiotics), non-digestible carbohydrates (dietary fibers and prebiotics), postbiotics (parts of probiotics) and bioactive plant secondary metabolites such as phenolic compounds. 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. The most commonly used probiotic bacterial genera are *Lactobacillus* and *Bifidobacterium*.

Besides probiotic, the new term “postbiotic” 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. 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 probiotic and postbiotics (e.g., yoghurt, kefir, pickled vegetables and kombucha). Due to technological advantages and favourable taste, milk has emerged as the most suitable medium for probiotic products. However, these products cannot be consumed by groups who suffer from lactose intolerance or have allergies to milk protein.

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 the functional health ingredients. 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) may influence the viability and activity of bacteria during fermentation and storage. Research now focuses on characterizing specific probiotic strains and the growth and survival of probiotic in fruit juices. An easy way to improve probiotic stability in fruit juice could be the fortification of juice with some prebiotics or some ingredients that can exert a protective effect. Another possibility of exposure of probiotics to a sub-lethal stress could induce resistance and an adaptive stress response.

Taste is the primary factor involved in the acceptance and purchasing behaviour of various foods, including functional foods. The off-flavour appears to be related to the presence of probiotics on the sensory characteristics of juices. Masking is one technique that has been used to reduce the sensations of aversive odours and flavours in foods. It has been performed successfully through the addition of new substances or flavours to juices and is therefore suspected to be capable of reducing the negative sensory attributes contributed by probiotic cultures. Tropical fruit juices (e.g., pineapple, mango, passionfruit) contribute strong, exotic aromatic and flavour contributions that may prevent consumers from identifying the probiotic off-flavours.

The development of new non-dairy probiotic food products was carried out by many researchers. The substrates that they mainly focused on were vegetables and fruits, such as soymilk, the juice of cucumber, potato, carrot or fruit juices (apricot, pineapple, mango, apple, etc.). Some promising results were reported from their studies. However, there are only limited studies on the combination of different probiotic microorganisms in these media. Mixed cultures were believed that can bring more effective health benefits than single strains. Furthermore, it would be interesting to compare the metabolism and growth rate of these bacteria in mono and mixed cultures. Additionally, since fermented fruit juices are known as a novel probiotic product, shelf-life evaluation is important to ensure a quality product during the storage period.

My PhD research focused on the development of probiotic tropical fruit juices by fermentation with probiotic bacteria *Lactobacillus* and *Bifidobacterium* strains.

The specific objectives were:

- Investigation of the suitability of some tropical fruit juices for probiotic fruit drink production applying lactic acid bacteria (LAB) and *Bifidobacterium*
- Screening probiotic strains for fermentation of fruit juices
- Production of fermented fruit juice with mixed cultures
- Investigation of viability and production of metabolites, as well as the change of total phenolic content and antioxidant activity of the juices during fermentation and storage
- The survival of probiotics through the simulated gastro-intestinal conditions
- Sensory analysis
- Effects of storage conditions on the stability of fermented products, estimation of the product shelf life.

2. MATERIALS AND METHODS

2.1. Fruit juices

Commercial pineapple, mango and banana juices were purchased from the local market. The juice pH was adjusted to pH 6.4 with 4 N NaOH before fermentation.

2.2. Microorganisms

Three *Lactobacillus* strains: *Lactobacillus acidophilus* 150 (Exquim SA, Spain), *Lactobacillus casei* 01 (Chr. Hansen, Denmark), *Lactobacillus plantarum* 299v (Probi, Sweden) and two *Bifidobacterium* strains: *Bifidobacterium lactis* Bb12 (Chr. Hansen, Denmark) and *Bifidobacterium longum* DSM16603 (Probiotical, Italy) were used.

The *Lactobacillus* strains were grown in MRS medium at 37^{o}C for 24 h and *Bifidobacterium* strains in TPY medium also at 37^{o}C for 24 h anaerobically in an anaerobic jar gas-pak system.

2.3. Chemicals

2.3.1. Media

De Man, Rogosa, and Sharpe (MRS) broth and **trypticase-phytone-yeast extract** (TPY) broth were applied.

MRS-bile agar was obtained by the addition of 0.3% bile salts into the MRS medium. Agar medium is the medium supplemented with agar in a concentration of 15 g/L.

2.3.2. Other chemicals

Folin-Ciocalteu reagent, tripyridyltriazine, Iron (II) sulfate, gallic acid, standards (glucose, fructose, lactic acid and acetic acid) as well as pepsin and bile salts were purchased from different companies.

2.4. Fermentation of fruit juices using monocultures

150 mL Erlenmeyer flasks containing 50 mL juices were inoculated with 1% of individual probiotic culture. The initial cell concentration in juices was around 6.5-7.5 log CFU/mL in the case of *Lactobacillus* strains, and around 6-6.5 log CFU/mL in the case of *Bifidobacterium* strains. The fermentations were conducted in an incubator for 16-24h at 37^oC. After incubation, the fermented fruit juices were stored at 4^oC for four weeks. The stability of the products during fermentation and storage was investigated through the changes of cell number, pH, quantity of acid and carbohydrates, total phenolic content and antioxidant activity. The survival of microorganisms through simulated gastro-intestinal conditions was also evaluated.

2.5. Fermentation of mixed fruit juice using monocultures

The probiotic bacteria screened from the previous experiment were used to ferment mixed fruit juice composed of an equal proportion of pineapple, mango and banana. 1% of each monoculture was added. All the flasks were placed into an incubator set at 37^oC for 16 hours. The samples with *Bifidobacterium* strains were put into an anaerobic jar before incubation. After fermentation, the samples were stored at 4^oC for 4 weeks.

2.6. Fermentation of fruit juices using mixed cultures

Individual juices of pineapple, mango and banana and mixed fruit juices with a combination of the three juices at different ratios were used to produce probiotic fruit drink products. The strains of bacteria were mixed in an equal proportion and then used as a starter for fermentation. The samples were then kept at 4^oC for 4 weeks.

2.7. Sensory evaluation

After fermentation, the fruit juices were refrigerated at 4^oC for one day before evaluation. Sensory analysis was carried out by seven panelists (3 females and 4 males), ranging in age from 25 to 45. The appearance, aroma, taste, texture and overall attributes of the fermented fruit juice formulations were chosen for acceptance testing using a 9-points hedonic scale (9-like extremely and 1-dislike extremely). The tests were performed in individual tables under white light.

2.8. Storage study

The influence of storage temperatures on the change of product quality was carried out through maintaining the products at different temperatures of 5°C, 15°C, 25°C and 35°C. Samples stored at 5°C were measured every 6 days at the initial storage time and every 3 days at the end of storage. The samples stored at 15°C were tested every 3 days. Those at 25°C were sampled every day, while samples stored at 35°C were evaluated every 12 hours due to their short shelf-life.

2.9. Analytical methods

2.9.1. Viability of probiotic strains

The viable cell number of *Lactobacillus* and *Bifidobacterium* strains were determined by pour plate method.

2.9.2. Measurement of Brix and pH

The total soluble solids (Brix) and pH were measured by using a refractometer and a pH meter, respectively.

2.9.3. Organic acid and sugar quantification

Carbohydrate and organic acid content were determined by HPLC method.

2.9.4. Total phenolic content

The content of total polyphenols in fermented juices was determined using Folin's phenol reagent method (ISO:14502-1:2005) which has some minor modifications.

2.9.5. Antioxidant capacity

The antioxidant activity was evaluated following ferric reducing antioxidant power assays.

2.9.6. Survival of probiotic strains through simulated gastro-intestinal conditions

The probiotics were exposed for 135 min in 0.5% NaCl (pH 2.0) containing pepsin in the concentration of 0.3%, followed 150 min incubation in the presence of 0.6% bile salts prepared in potassium phosphate buffers (pH 7.4). The survival rate of microorganism was calculated and evaluated.

2.10. 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 statistically significant difference between the variables.

3. RESULTS AND DISCUSSION

3.1. Fruit juice fermentation with monoculture

3.1.1. Change of viability and pH

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* strains 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. For *Bifidobacterium* strains, after 24 h of fermentation, the pH values of pineapple juice decreased from pH 6.4 to 3.8. Fermented mango and banana juice demonstrated a slightly higher pH value (approx. pH 4.1) than that in fermented pineapple juice. Interestingly, the juices inoculated with *B. lactis* Bb12 showed a slighter decrease in pH value during the fermentation period from hour 8^{th} to 20^{th} compared to *B. longum* DSM16603. However, the rising fermentation time minimized the gap between the pH values in the fermented juices. During storage, the pH in fermented juices slightly decreased in the cases of *Lactobacillus* and *Bifidobacterium* strains.

All *Lactobacillus* and *Bifidobacterium* strains could grow well in the investigated juices without any supplementation of nutrients. The microbial population in the juices after fermentation was not lower than 8 log CFU/mL. For the *Lactobacillus* strains, the juices inoculated with *L. casei* 01 and *L. plantarum* 299v presented the highest viable cell counts (approx. 10 log CFU/mL in pineapple juice, and approx. 9 log CFU/mL in mango and banana juice). The *L. acidophilus* 150 strain showed the poorest growth in fruit juices. In the case of *Bifidobacterium* strains, although *B. lactis* Bb12 showed slighter growth than *B. longum* DSM16603 at initial fermentation time (hour 0^{th}-12^{th}), the population of these strains were similar at the end of fermentation period. The viable cell counts reached approx. 10 log CFU/mL in pineapple juice and 9 log CFU/mL in mango and banana juice. During 4 weeks of storage at 4°C, most bacteria remained stable in population.

3.1.2. Change of sugar and organic acid

Mango juice 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.

Both glucose and fructose decreased while organic acid (lactic and acetic acid) increased during fermentation and storage process. In the cases of *Lactobacillus* strains, after 16 hours of fermentation, sugar content in pineapple juice decreased by around 1.6% w/v in glucose and 0.6% w/v in fructose. In mango juice, the concentration dropped by in a range of 1.6-2.9 (% w/v) in glucose and in a range of 0.8-1.2 (% w/v) in fructose. *Lactobacillus* strains utilized around 1.3% w/v glucose and 0.6% w/v fructose in banana juice after fermentation. Glucose was observed as the preferable carbohydrate source for these strains. *Bifidobacterium* strains utilized both glucose and fructose for their metabolism. *B. longum* DSM16603 used 1.96% w/v glucose and 2.1% w/v fructose for pineapple juice fermentation, 2.01% w/v glucose and 0.8% w/v fructose for mango fermentation and 1.14% w/v glucose and 2.19% w/v fructose for mango fermentation. Meanwhile *B. lactis* Bb12 consumed 1.8% w/v glucose and 1.21% w/v fructose in pineapple, whereas 1.32% w/v glucose and 0.59% w/v fructose in mango and 1.45% w/v glucose and 0.66% w/v fructose in banana juice. These results indicated that higher amounts of sugars were consumed by *B. longum* DSM16603 than *B. lactis* Bb12. During storage, all the investigated strains kept using glucose and fructose for growth.

Organic acids were accumulated during carbohydrate metabolism of LAB and bifidobacteria. After 16 h of fermentation, the lactic acid concentration of juices was in a range of 1.7-2.01 (% v/v) in pineapple juice, 0.9-1.39 (% v/v) in mango juice and 1.47-1.77 (% v/v) in banana juice. Fermented pineapple juice had the highest lactic acid concentration in all investigated juice. The lactic acid concentration of pineapple juice inoculated with *Lactobacillus* strains was in a range of 2.01-2.85 (% v/v) in pineapple juice, 1.23-1.75 (% v/v) in mango juice and 1.59-1.93 (% v/v) in banana juice after storage for 4 weeks. The lactic acid concentration of juices increased during fermentation with *Bifidobacterium* strains. In detail, the lactic acid content of fermented pineapple was 2.7-3.06 (% v/v). A lower value was recorded in the case of mango juice and banana juice which were in a range of 1.75-2.09 (% v/v) and 1.33-1.77 (% v/v), respectively. Lactic acid concentration of fermented pineapple juice continued increasing during storage and reached 2.97-3.44 (% v/v) at the end while the quantity of this acid remained unchanged in mango and banana juice. Besides lactic acid, acetic acid was also reported as a product of sugar metabolism. However, the concentration of this acid in the fermented tropical fruit juices was quite low ranged in 0.04-0.21 (% v/v) stable during storage.

3.1.3. Change of total phenolic content and antioxidant capacity

In the present study, total phenolic content (TPC) and antioxidant activity (FRAP) of juices showed a slight decrease during fermentation and a significant reduction during storage. The pineapple juice had the highest TPC value around 0.45-0.46 (µg/mL gallic acid), followed by mango juice with 0.38-0.4 (µg/mL gallic acid) and banana juice with 0.33-0.37 (µg/mL gallic acid). In the juices inoculated with *Lactobacillus* strains, TPC content decreased by approx. 1.4%-14.2% after fermentation and reached 7.8%-26.26% after storage. In the case of the juices fermented with *Bifidobacterium*, TPC concentration decreased more significantly than with *Lactobacillus*. These values dropped by 8.28%-22.54% after fermentation and 11.36%-31.63% after storage.

The highest FRAP value was observed in mango juice (4.05 mM $FeSO_4$), followed by banana (3.49 mM $FeSO_4$). The antioxidant activity of pineapple juice was the lowest with 2.55 mM $FeSO_4$. After 16 h of fermentation, the antioxidant activity of samples with *Lactobacillus* strains dropped by approx. 1.6%-13.9%. After four weeks of storage at 4°C, antioxidant capacity of fermented pineapple juice decreased in a range of 14.32%-21.04%. A more significant decrease in FRAP was observed in mango and banana juice. The FRAP values reduced 16.28%-31.65% in the case of mango juice and 27.28%-42.76% in banana juice at the end of storage period. The FRAP values of juices reduced by 10.89%-23.82% after 24 h of fermentation with *Bifidobacterium* strains. After storage, FRAP values of pineapple, mango and banana juice dropped by 12.1%-18.07%, 33.98%-44.86% and 28.15%-36.63%, respectively.

3.1.4. The survival of bacteria through the simulated gastro-intestinal model

The effect of storage (4^{o}C for 4 weeks) on survival of *Lactobacillus* and *Bifidobacterium* strains in fermented tropical fruit juices through the simulated gastro-intestinal conditions was conducted. The survival of *Lactobacillus* strains in all fermented fruit juices after exposure to pepsin and bile salts solution was about > 80%. The similar trend was found in the case of *Bifidobacterium* which obtained the survival through simulated gastro-intestinal conditions except *B. lactis* Bb12 in banana juice which had lower survival rates (86.2% in pepsin and 69.07% in bile salts). However, after storage for four weeks, their survival was improved reached 99.48% and 93.11%, respectively. *B. longum* DSM16603 in mango juice showed an opposite trend. 70.69 % and 56.42% of survival of *B. longum* DSM16603 through pepsin and bile salts solution were observed, respectively after storage for 4 weeks.

3.2 Fermentation of mixed fruit juice using monocultures

Mixed fruit juice of pineapple, mango and banana in equal proportion was fermented using single strain of *L. casei* 01, *L. plantarum* 299v and *B. lactis* Bb12. The fermentation was carried out in an incubator at 37^{o}C for 16 h, and then stored at 4^{o}C for 4 weeks.

3.2.1 Change of viability and pH

The three probiotic bacteria, *L. casei* 01, *L. plantarum* 299v and *B. lactis* Bb12 strains grew rapidly in the juices and reached around 9 log CFU/mL after 16 h of fermentation. The *L. casei* 01 strain had the highest microbial population in the juice after fermentation. Storage did not result in a decrease in viable cell counts of these strains.

A declining trend was observed in pH value that dropped from pH 6.4 at initial to around pH 4.3 at the end of the fermentation. The lowest value was recorded in the case of juice fermented with *Lactobacillus* strains (around pH 4.0). The decrease rates in pH of juices inoculated with *Bifidobacterium* strain were significantly slower than the juices with *Lactobacillus* strains. After 16 h of fermentation, the pH of the juice containing *B. lactis* Bb12 was pH 4.35.

The growths of *Lactobacillus* and *Bifidobacterium* strains in the mixed juice of pineapple, mango and banana were better than in single mango or banana juice. Additionally, the substrate shortened the fermentation time of *B. lactis* Bb12 from 24 h to 16 h based on the pH value (<4.5).

3.2.2. Change of sugar and acid content

Glucose and fructose contents of the juice were 7.29% w/v and 3.39% w/v, respectively. Upon completion of fermentation, the concentration of glucose and fructose dropped by in a range of 0.53% w/v-1.09% w/v and in a range of 0.22% w/v-0.6% w/v, respectively. The decreases in the sugar contents were also observed during the storage period.

The production of lactic acid was more intensive in the juices fermented with *Lactobacillus* strains (around 2.2% v/v) compared to *Bifidobacterium* strain (1.78% v/v). The highest lactic acid concentration was observed in the case of *L. casei* 01 (3.84% v/v), followed by *L. plantarum* 299v (3.33% v/v) and *B. lactis* Bb12 (2.73% v/v), after storage for 4 weeks.

3.2.3. Change of total phenolic content and antioxidant activity

The initial phenolic content of the juice was 0.37 µg/mL gallic acid, and the antioxidant activity was 3.81 mM $FeSO_4$. These parameters decreased during fermentation. After fermentation, a lower value of FRAP was recorded in the juice inoculated with *B. lactis* Bb12 compared to the juice with *Lactobacillus* strains. In detail, the FRAP value of juice fermented with *B. lactis* Bb12 was 3.16 mM $FeSO_4$, with both *Lactobacillus* strains was around 3.47 mM $FeSO_4$. A slight continuous decline of TPC and FRAP was observed during storage.

3.3. Fruit juice fermentation with mixed culture

Fermentation of each fruit juices (pineapple-P, mango-M, banana-B) and their mixture with ratios of 70P:15M:15B, 50P:25M:25B and 33P:33M:33B with mixed culture of *L. casei* 01*:L. plantarum* 299v*:B. lactis* Bb12 (1:1:1) were conducted at 37^{o}C for 16 hours, and then stored at 4^{o}C for 4 weeks.

3.3.1. Change of viability and pH

After 16 h of fermentation, meanwhile pH values of all mono fruit juices dropped slightly from pH 6.5 to pH 4.25, pH 4.33 and pH 4.49, respectively, whereas bigger decrease in pH values (to pH 4) of the mixed samples were observed. The pH values of all fermented juices continued the decline tendency during storage, and reached pH 3.51, pH 3.64 and pH 3.76 of fermented pineapple, mango and banana, respectively

The total microbial viability in most fermented substrates after fermentation obtained in a range of 9.18-9.41 (log CFU/mL), except in banana juice with lower viable cell count of 8.92 log CFU/mL. These results illustrated that mixed culture could improve the growth of each microorganism in all fruit juices and mixed juices. The microbial population in mixed fruit juices with the ratios of 70P:15M:15B and 50P:25M:25B were higher than in other combinations.

3.3.2. Change of sugar and organic acid

An increase in organic acid content was observed in all the media during fermentation and storage. After fermentation, the highest concentration of lactic acid was recorded in the case of mixed fruit juice (2.68% v/v) with ratio of 70P:15M:15B, followed by pineapple juice (2.33% v/v), mixed juice with ratio of 50P:25M:25B (2.30% v/v), mixed fruit juice with ratio of 33P:33M:33B (2.03% v/v) and banana juice (0.98% v/v). Mango juice had the lowest quantity of lactic acid concentration (0.56% v/v).

The concentration of lactic acid rose significantly during storage for 4 weeks. At the end of the storage period, mixed fruit juice with ratio of 70P:15M:15B and pineapple juice obtained the highest lactic acid concentration (approx. 4.2% v/v). The lactic acid contents of fermented mango and banana juices were less than half of the concentration of others (1.67% v/v-1.82% v/v).

Acetic acid was detected in small amount in all fermented juices and mixed juices. Higher acetic acid concentration was measured in the individual pineapple, mango and banana juices than the mixed fruit juices. The highest concentration was observed in the case of pineapple juice, and the values were 0.11% v/v and 0.16% v/v after fermentation and storage, respectively. Very small quantity of acetic acid was detected in the cases of mixed juices.

Sharp declines of glucose and fructose concentration were observed in all juices and mixed juices. After 16 h of fermentation, the glucose and fructose content in pineapple juice decreased by 1.42% w/v and 0.9% w/v, respectively. In the mixed juice with ratio of 70P:15M:15B, these values were 1.66% w/v and 1.81% w/v, respectively. In the mixed juice with ratio of 50P:25M:25B, glucose concentration reduced by 2.8% w/v and fructose content dropped by 1.52% w/v. In the mixed juice with ratio of 33P:33M:33B, glucose and fructose quantity decreased by 1.24% w/v and 0.73% w/v, respectively. Meanwhile, in mango juices, these values were 0.55% w/v and 0.42% w/v, whereas in banana juice, they were 1.14% w/v and 0.24% w/v, respectively.

During storage process, the glucose and fructose concentration of the fermented juices continued decreasing. After fermentation and storage, the probiotic bacteria used 2.13% w/v-3.63% w/v glucose and 1.11% w/v-1.96% w/v fructose. Lower sugar consumptions (0.78% w/v-1.26% w/v glucose and 0.35% w/v-0.57% w/v fructose) were recorded in mango and banana juices.

3.3.3. Change of total phenolic content and antioxidant activity

The total phenolic content in these media decreased during fermentation and storage. After fermentation, the highest decline (15.4%) in TPC was observed in the case of mango juice, followed in the cases of pineapple juice, banana juice and the combination of 50P:25M:25B (approx. 11%). The TPC of mixed juices with ratio of 70P:15M:15B and 33P:33M:33B decreased by 9.08% and 5.08%, respectively. During storage, the concentration of TPC kept reducing slightly. Fermented mango juice registered as the highest decline in TPC (19.57%), while the mixed fruit juices with ratio of 33P:33M:33B was recorded as the lowest (10.01%).

The highest FRAP values were observed (approx. 3.6 mM $FeSO_4$) in mango, banana juice and mixed fruit juice with ratio of 33P:33M:33B, whereas the smallest value was in pineapple juice (2.73 mM $FeSO_4$). Antioxidant capacity dropped significantly after first four hours of fermentation in all mono and mixed juices. From the hour 4, this parameter decreased gradually until the end of the storage period in the cases of mango, banana and mixed fruit juices with ratio 33P:33M:33B as well as unchanged sharp in the cases of pineapple, mixed fruit juices with the ratios of 70P:15M:15B and 50P:25M:25B. After storage, the highest decrease of FRAP was observed in banana juice (28.64%), followed ones in the mixed fruit juices (70P:15M:15B and 33P:33M:33B) (approx. 17.5%), in mango juice (16.15%) and in pineapple juice (13.78%). In the case of the mixed fruit juice with ratio 50P:25M:25B, the lowest change in FRAP (7.2%) was recorded.

3.3.4. Probiotic survival through the simulated gastro-intestinal model

Mixed culture was not significantly affected by artificial gastric fluid and bile salt through the model. The survivals of *Lactobacillus* and *Bifidobacterium* strains were over 90% after storage at 4^oC for 4 weeks. The results indicated that the bacteria would not enhance the sensitivity to gastric fluid and bile salt of each other when growing in the media simultaneously.

3.3.5. Sensory evaluation

The acceptance of sensory attributes (appearance, aroma, taste, texture and overall) of fermented fruit juices was carried out. The consumer accepted most of the formulations in the range of like moderately to like very much (7-8 points) except fermented pineapple juice with overall score of 6.43 points (based on hedonic scale of 9 points). Consumers highly accepted the fermented mango and banana juices while pineapple juice had the lowest acceptance. Both attributes received moderately liked scores (approx. 7 points). The taste did significantly influence the preference ranking of fermented juices in this experiment. Pineapple juice came with the lowest acceptance score of taste (6.71 points). The most preferred in taste was registered for the banana with 8.29 points, and there was no significant different between mango (7.86 points), mixed fruit juices with ratios of 50P:25M:25B (7.86 points) and 33P:33M:33B (7.29 points)

3.4. Storage study

The mixed fruit juice (pineapple:mango:banana) with ratio of P50:M25:B25 was used for storage study. After fermentation with mixed culture of *L. casei* 01*:L. plantarum* 299v*:B. lactis* Bb12 (1:1:1) at 37°C for 16 hours, the products were stored at different temperatures including 5°C, 15°C, 25°C and 35°C until they begin to failure (pH<3.4)

3.4.1. Change of cell number

During the storage, the samples showed a reduce tendency in pH value. The pH dropped from pH 3.94 at the initial storage time to around pH 3.4 at the end. At this pH value, the product may not be accepted sensorially. Significant decline of pH was observed at the beginning, and then the decrease was followed at lower rate and remained almost unchanged at the later stage (in the cases of 35°C and 25°C). However, lower temperatures may cause a delay of acidification and lower rates of pH decline (in the case of 15°C and 5°C).

The viable cells showed different trends depending on the storage temperature. The bacteria kept a gradual increase in number from 9.43 log CFU/mL at beginning to approx. 9.5 log CFU/mL during three days in the case of 25°C and 1.5 days in the case of 35°C. Then, it declined significantly to 9.36 log CFU/mL and 9.19 log CFU/mL at the end of storage, respectively. When storage at the lower temperature (5°C and 15°C), the microbial population had slight decrease at the beginning of storage period and slight increase afterwards. In the case of storage at 15°C, after 3 days with a decline tendency in population, the microbial viability started increasing and reached 9.55 log CFU/mL at the end of storage (18 days). In the case of storage at 5°C, very small change of cell number was recorded.

3.4.2. Change of pH, organic acid and sugar

During the storage, pH values showed decrease from pH 3.94 at the initial storage to approx. pH 3.4 at the end. In the cases of storages at 25°C and 35°C, the pH values declined significantly at the beginning of storage period, and then slowly. In the cases of storage at lower temperatures (5°C and 15°C), the pH values declined gradually.

The drop of pH during storage is due to the acid accumulation. At the higher temperature, the rate of increase in lactic acid level was greater than that at the lower temperature. In particular, while the lactic acid concentration gradually increased by 39.11% after 45 storage days at 5°C, whereas it increased sharply by 47.11% after 3.5 days of storage at 35°C. At 15°C and 25°C, both products showed similar increase rates in lactic acid level during the first 6 days. Afterwards, the lactic acid concentration increased by 39.11% after 7 days of storage in the case of storage at 25°C, and by 44% at 15°C for 18 days. The acetic concentration increased slowly at low temperatures (5°C and 15°C). At higher temperatures (25°C and 35°C), a rapid increase in acetic acid level was recorded. After storage, the acetic acid concentration of the samples reached the range of 0.07% w/v-0.13% w/v.

The sugar concentration (glucose and fructose) of all products decreased during storage. When storage at 25°C and 35°C, glucose and fructose contents of these products dropped significantly at the beginning, then it was followed by slower rate. At the end of storage, the concentration of glucose and fructose decreased by approx. 46% and 25%, respectively. At 5°C and 15°C, both the products displayed a gradual decrease in glucose and fructose levels at the beginning and remained almost constant in the later stage. The concentration of glucose and fructose decreased in the range of 38.4%-44.4% and 17.8%-23.1%, respectively.

3.4.3. Change of total phenolic content and antioxidant capacity

Storage condition affected significantly on TPC and FRAP of fermented mixed fruit juices. Before storage, the concentration of TPC compounds and FRAP capacity of fermented mixed fruit juice were 0.42 µg/mL gallic acid and 3.49 mM $FeSO_4$, respectively. After storage, TPC value decreased by 8.06%-10.4% and FRAP dropped by 11.17%-34.05% varied on storage temperature conditions. Fermented fruit juice stored at 25°C and 35°C had the lowest decreases in these parameters (11.17% and 8.06%, respectively). There was no significant difference in the TPC value when storage at 15°C, 25°C and 35°C. However, the antioxidant activity of the fermented mixed juice stored at 15°C was significantly lower than those at 25°C and 35°C. The highest lost in TPC and FRAP values were recorded in the case of 5°C (10.4% and 34.05%, respectively).

3.4.4. Estimation of shelf-life of beverages

The shelf-life of fermented fruit juice products in the current study was established based on the change of pH values. The regression equations of several models: zero-order, first-order, second-order and third-order were obtained. These regressions show that in zero- and first-order models, the slope of equations decreased against the increase of storage temperature while in the second- and the third-order, the slope increase with the rising temperature. Based on the coefficient of determination (R^2), the third order model with the highest R^2 value (over 0.8) was in accordance with the pH kinetic. Therefore, the shelf-life of fermented fruit juice was predicted using third order model.

Based on the Arrhenius equation, the regression of rate constant against at temperatures was obtained (**Eq. 3.1**)

$$Lnk = -7317.2\frac{1}{T} + 17.881 \text{ with } R^2 = 0.9941 \quad \textbf{(Eq.3.1)}$$

Based on the third order reaction and Arrhenius equation (**Eq. 3.1**), the shelf-life kinetic model of change in pH value was described as **Eq. 3.2**

$$\frac{1}{pH^2} = 1.1658 \times 10^8 \times e^{\frac{-7317.2}{T}} \times t + \frac{1}{pH_o^2} \qquad \textbf{(Eq. 3.2)}$$

where, pH is the expected pH value at the end of storage period, T is the storage temperature (K), t is the storage time (days) and pH_o is the initial pH value of fermented juice before storage

The root mean square error (RMSE) was calculated to evaluate the fit of the model using sets of data from experimental tests and model prediction of the product's shelf-life stored at 30°C. The initial pH of fermented fruit juice is pH_o = 4.09, and the final pH was pH 3.4. The shelf-life of the sample was predicted as t = 7.05 days (obtained from **Eq. 3.2**). The predicted and experimental data were checked**,** and strong correlation was observed.

At 5°C, the shelf-life of fermented juice was predicted to be 51.1 days using the **Eq. 3.2.** Similarly, the shelf lives of the fermented juices stored at 15°C, 25°C and 35°C were estimated to be 20.5 days, 8.7 days and 3.9 days, respectively.

4. CONCLUSION AND RECOMMENDATION

In this study, pineapple, mango and banana juices were demonstrated to be good media for the growth of probiotic bacteria without any nutrient supplementation. Pineapple juice was observed as the most suitable medium compared to other substrates. All investigated *Lactobacillus* strains and *Bifidobacterium* strains could grow and maintain their viability (over 8 log CFU/mL) in the tropical juices during fermentation and storage. The *L. casei* 01, *L. plantarum* 299v and *B. lactis* Bb12 strains were promising cultures for production of probiotic tropical fruit drinks. The products were well accepted by consumers except the fermented pineapple juice. The shelf life of product was estimated with the fermented mixture of pineapple, mango and banana with ratio of 50:25:25 and mixed culture of probiotic *L. casei* 01*:L. plantarum* 299v*:B. lactis* Bb12 (1:1:1) to be 51.1 days at 5°C.

This study provided promising results for the development of new non-dairy probiotic food products. Some directions for further research can be proposed as follows:

- Complementary studies on the impact of the fermentation process on the formation of aroma compounds and bioactive compounds produced by probiotic bacteria.
- Experimental results showed that pineapple juice was the most suitable substrate for all the investigated probiotic strains. This is likely due to the presence of some components in the juice which may confer positive effects on the growth of bacteria. Deeper research is recommended to discover such components.
- Scaling up, optimisation as well as comprehensive sensorial evaluation are needed for adaptation of these results in production of commercialised probiotic fruit juices.

5. NOVEL CONTRIBUTIONS

1. Pineapple, mango and banana juices are as good as media for growth of three probiotic *Lactobacillus* strains (*L. acidophilus* 150, *L. casei* 01 and *L. plantarum* 299v), and two probiotic *Bifidobacterium* strains (*B. lactis* Bb12 and *B. longum* DSM16603) without any supplement of nutrients. The *L. casei* 01, *L. plantarum* 299v and *B. lactis* Bb12 strains were promising cultures for production of probiotic tropical fruit drinks.
2. The survival of *Lactobacillus* and *Bifidobacterium* strains in all fermented fruit juices after exposure to pepsin and bile salt solution was over 80%. Storage condition did not affect significantly on the survival of these bacteria except in the case of *B. longum* DSM16603 in mango juice.
3. The fermented beverage products including mango, banana juices and mixed juices with ratio of 70P:15M:15B, 50P:25M:25B and 33P:33M:33B were well-accepted by the panelists in the range of like moderately to like very much.
4. The mixed culture demonstrated a good viability after stored at different temperatures (5°C, 15°C, 25°C and 35°C). The rate of acid increase (lactic and acetic) and sugar decrease (glucose and fructose) linked with the increase of the storage temperatures. Slight decrease in total phenolic contents and antioxidant activity values during fermentation and storage were observed.
5. The third order model and Arrhenius equation were adequate for the prediction of shelf lives of the fermented fruit juices based on the changes of pH during storage. The shelf lives of probiotic drinks were estimated to be 51.1 days, 20.5 days, 8.7 days and 3.9 days when stored at 5°C, 15°C, 25°C and 35°C, respectively.

6. PUBLICATIONS

Journal articles

1. **Nguyen, B. T.**, Bujna, E., Fekete, N., Tran, T. M. A., Rezessy-Szabó, J., Prasad, R., Nguyen, D. Q. (2019): Probiotic beverage from pineapple juice fermented with *Lactobacillus* and *Bifidobacterium* strains. *Frontiers in Nutrition,* 6: 54
2. Tran, T. M. A., **Nguyen, B**. **T.**, Nguyen, D.V., Bujna, E., Dam, S. M., Nguyen, D. Q. (2020) Changes in bitterness, antioxidant activity and total phenolic content of grapefruit juice fermented by *Lactobacillus* and *Bifidobacterium* strains. *Acta Alimentaria,* 49:103-110
3. **Nguyen, B. T.**, Bujna, E., Wheize, S., Szécsi, A., Rezessy-Szabó, J., Pham, M. T., Hitka, G., Friedrich, L., Usmani, Z., Sharma, M., K. Gupta, K. V., Nguyen, D. Q. (2021): Storage stability of probiotic mango juice fermented with Lactobacillus and Bifidobacterium. *Food Chemistry* (**under reviewing process**)
4. Pham, M. T, Varjú, R., Bujna, E., Hoschke, Á., Csernus, O., **Nguyen, B. T.**, Gupta, K. V., Nguyen, D. Q. (2021): Chemical and volatile composition of fermented apple juice with different commercial yeast strains of Saccharomyces cerevisiae. *International Journal of Food Microbiology* (**under reviewing process**)

Poster presentations

1. **Nguyen, B. T.**, Bujna, E., Nguyen, D. Q. (2021): Estimation of shelf-life of probiotic fruit juice by using physicochemical change during storage. *The 4th International Conference on Biosystems and Food Engineering*.
2. **Nguyen, B. T.**, Bujna, E., Nguyen, D. Q. (2020): Suitability of some tropical fruit juices for the production of probiotic product fermented by *Bifidobacterium lactis* Bb12. *Ifjú Tehetségek Találkozója*.
3. **Nguyen, B. T.**, Bujna, E., Tran, T. M. A., Nguyen, D. Q. (2019): Effect of fermentation of mango juice by some lactic acid bacteria on the antioxidant activity and phenolic compound. *18th International Congress of The Hungarian Society for Microbiology*.
4. **Nguyen, B. T.**, Bujna, E., Tran, T. M. A., Nguyen, D. Q. (2018): Storage stability of pineapple juice fermented by probiotic bacteria *Lactobacillus* sp. *3rd International Conference on Food Science and Technology*.
5. **Nguyen, B. T.**, Bujna, E., Tran, T. M. A., Nguyen, D. Q. (2018): Fermentation of pineapple juice by some probiotic *Lactobacillus sp. Fiatal Biotechnológusok Országos Konferenciája (FIBOK)*.