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Research Article

Survival of *L. casei* BD II and *L. plantarum* WCFS1 in gastro-intestinal stresses and viability in mango juice during storage

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Abstract

Mango fruit (*Mangifera indica L.*) is a good source of beneficial nutrients and has been proven to have prebiotic potential. In addition, recent studies on novel probiotic strains *Lactobacillus casei* BD II and *Lactobacillus plantarum* WCFS1 suggest its applicability in the food industry. In this study, we report the suitability of mango as a raw material for production of a non- dairy probiotic beverage and the resistance of *L. casei* BD II and *L. plantarum* WCFS1 to gastro-intestinal (GI) stresses. Prior to inoculation in mango juice (MJ), *Lactobacillus* strains were examined for their survival in acidic conditions (pH 1.0 and 3.0), presence of bile and digestive enzymes pepsin (at pH 2.0) and pancreatin (at pH 8.0). *L. plantarum* WCFS1 was found to be more tolerant at pH 1.0 and 3.0 at 0, 1, and 3 h as well as in the presence of digestive enzymes pepsin and pancreatin at 0 and 4h, compared to *L. casei* BD II ($p<0.001$); both strains were found to be tolerant against bile salts and showed growth in a modified media (1.0% w/v bile salts) after 48 h at 37°C. MJ containing 30.00% (w/v) mango puree was then inoculated with the two strains of lactic acid bacteria and stored at 25 ± 2°C and 4°C up to 4 weeks. Cell viability, change in total acidity computed as % lactic acid production and change in pH were monitored at weekly intervals. It was concluded that *L. casei* BD II and *L. plantarum* WCFS1 were able to rapidly utilize MJ for cell synthesis and lactic acid production without pH adjustment or addition of other nutrients and remained viable (≥10^6cfu ml^{-1}) up to 3 weeks and 4 weeks of storage at 4°C. Therefore, microencapsulation of the probiotic cells is highly recommended to enhance its viability when inoculated into fruit juice.

Keywords: *Lactobacillus plantarum* WCFS1, *Lactobacillus casei* BD II, mango juice, probiotics, Philippines.

Introduction

The prevalence of lifestyle-related diseases has encouraged consumers to seek healthier alternatives among food commodities. Development of ordinary food with functional ingredients, such as prebiotics and probiotics, is a convenient way of fulfilling market demand. Currently there is an ongoing trend in the food industry to create products, particularly beverages, containing health-promoting bacteria [1], [2], and[3].

Probiotics are live microorganisms with certain health- beneficial properties that affect the host when taken in proper amounts [4]. Although generally added in dairy products, probiotic cultures can also be incorporated in fruit and vegetable matrices as these media contain beneficial nutrients, such as minerals, vitamins, dietary fibres and antioxidants. Fruit juice may serve as carriers of good bacteria, if certain provisions regarding sensory characteristics and pH are considered [5]. However, probiotics must not only remain viable in the food during storage but it should also survive the gastro- intestinal transit in the digestive system to be able to exert their beneficial properties to the host [6] and [1].

Mango (*Mangifera indica L.*) is an accepted nutritious fruit worldwide due to its succulence, sweet taste and exotic flavor. Mango fruit is considered as a natural functional food [7] and has been referred to as the 'king of fruit'[8]. The mango pulp is a good source of dietary fibre in the form of pectic oligosaccharides (POS), which have been proven to have prebiotic potential [9]. Thus, mango juice may be used as a substrate for *Lactobacillus* strains and is an ideal raw material in developing a non-dairy fermented beverage.

Lactobacillus casei BD II was originally isolated from a traditional fermented alcoholic beverage in China and Mongolia called "koumiss" [10]. Recent studies on *L. casei* BD II showed that it has health benefits such as cholesterol-reducing effects, immuno-modulating effect [11] and antagonism to *Escherichia coli* in mice [12].

Meanwhile, the recent determination of the complete genome of *Lactobacillus plantarum* WCFS1, a single colony isolated from *L. plantarum* NCIMB 8826 from human saliva, validated that it has the coding capability for the uptake and utilization of several different sugars, uptake of peptides, and formation of most amino acids [13]. Due to its numerous surface-anchored proteins, *L. plantarum* WCFS1 has the potential to grow in a broad range of surfaces and substrates. In addition, majority of the genes encoding surface proteins exhibit homology to proteins with predicted roles, such as mucus-binding, aggregation-promoting and intracellular adhesion and has been verified to be safe for human consumption [14]. *L. casei* BD II and *L. plantarum* WCFS1 are novel probiotic strains that have the potential for food applications.

In this study we report the resistanceof *L. casei* BD II and *L. plantarum* WCFS1 to gastro-intestinal (GI) stresses brought by acidic conditions, presence of bile and digestive enzymes, as well as the suitability of mango as a raw material for production of non-dairy probiotic beverage.

Materials and Methods

Bacterial strains and growth conditions

Microbial isolates (*L. casei* BD II and *L. plantarum* WCFS1) used in this study were procured from the Department of Science and Technology, Microbiology and Genetics Division (DOST, Philippines). Identities of test isolates were verified using 16s rDNA analysis through Macrogen, Korea.

Survival in the simulated gastro-intestinal conditions

Resistance to low pH environment

Survival in low pH environment was determined using the method described by Conway *et al.* [15]. Bacterial cells from overnight cultures (17 h) were harvested by centrifugation at maximum speed (5 min), washed twice with phosphate buffered saline (PBS) solution (Sigma, Sigma- Aldrich, Inc., USA), pH 7.2, before being re-suspended in PBS solution, adjusted to pH 1.0 and pH 3.0. Resistance to low pH environment was assessed in terms of viable colony counts enumerated after incubation at 37°C for 0, 1, and 3 h, reflecting the time spent by food in the stomach.

Resistance to pepsin and pancreatin

Resistance to pepsin and pancreatin were tested according to the method by Charteris *et al.*, [16]. Bacterial cells from overnight cultures (17 h) were harvested by centrifugation at maximum speed (5 minutes), washed twice with PBS buffer, pH 7.2, before being re-suspended in PBS solution, pH 2.0, containing pepsin (3 mg ml^{-1}; Sigma, Sigma- Aldrich, Inc., U.S.A.), or in PBS solution, pH 8.0, containing pancreatin (1 mg ml^{-1}; Sigma). Pepsin and pancreatin solutions were subjected to direct Millipore filtration with 0.20 µm Acrodisc syringe filter prior to use. Resistance to the digestive enzyme pepsin was assessed in terms of viable colony counts enumerated after incubation at 37°C for 0, 1, and 3 h in pepsin solution; whereas, resistance to the digestive enzyme pancreatin was assessed in terms of viable colony counts enumerated after incubation at 37°C for 0 and 4 h in pancreatin solution, reflecting the time spent by food in the stomach and small intestine, respectively.

Tolerance to bile salts

Tolerance to bile salts was tested using the method as previously described by Quinto *et al.*, [17]. Ox gall powder (Spectrum Chemical Mfg. Corp., USA), was added to ROGOSA agar (Merck-Chemicals, Germany), at a final concentration of 1.0% (w/v), sterilized and plated. The test isolates were streaked on this modified medium and incubated at 37°C for 48 h and observed for growth. Isolates that showed positive growth on the special medium were considered to be bile tolerant strains.

Maintenance of test isolates

Purified test isolates of *L. casei* BD II and *L. plantarum* WCFS1 were stored in ROGOSA agar (Merck) tubes and plates and refrigerated at 4°C for future use. Frequencies of transfer were performed on a weekly basis for those stored on ROGOSA plates and on a monthly basis for strains stored in ROGOSA tubes.

Preparation of juice samples

Fresh ripe mangoes (*Mangifera indica L.*) purchased from a local market were used in the production of mango juice (MJ). MJ was prepared following the FAO and Codex Alimentarius standards. A batch formulation of MJ was comprised of the following food- grade ingredients: purified water, mango puree, refined sugar, citric acid (E- 330), ascorbic acid (E- 300), xanthan gum (E- 415) and artificial flavouring. The percentage recovery for a batch formulation ranged from 35 to 41%.

Mango puree and water were mixed together and the total soluble solids was adjusted to 16° Brix using an Atago type 500 hand refractometer (Atago, Tokyo, Japan). The pH was adjusted to 3.9 using a Milwaukee pH 600 meter (Milwaukee Meters, USA) by adding citric acid as 50% (w/v) solution. Sweetener, ascorbic acid, stabilizer and flavouring were added prior to thermal pasteurization of MJ. MJ was pasteurized at 90°C for 1 min then bottled and sterilized in chlorinated water bath (20 ppm) at 100°C for 15 min then cooled (4°C) for another 30 min prior to experimentation.

Formulated MJ was subjected to proximate analyses (Sentrotek Corp., Philippines) and was found to contain (g/100g) 83.65 moisture, 0.10 ash, 0.04 protein, 16.10 carbohydrate, 0.19 fibre and 0.08 fat. Its initial pH was 3.9, total soluble solids of 16 °Brix and titratable acidity of 0.21%.

Inoculation of lactic acid bacteria into mango juice

Lactobacillus strains were reactivated by sub-culturing twice in MRS broth, overnight (17 h) at 37°C. All cultures were harvested by centrifugation (10 min). The pellets were washed twice in 1.0% (w/v) normal saline solution (NSS) and concentrated ten-fold in the same diluent. 1.0% (v/v) inoculum of each lactic acid culture was distributed into the juice samples to obtain a final concentration of ~10^6cfu ml^{-1}[2].

Storage conditions of inoculated mango juice

Forty ml portions of inoculated MJ were stored in sterile plastic containers (Axygen, Axygen Inc., USA) were shaken vigorously using a vortex (5 min), sealed and placed inside airtight containers and stored at ambient (25 ± 2°C) and refrigerated temperature (4°C).

Microbiological and chemical analyses during storage of inoculated MJ

Survival of probiotic strains in MJ were determined in terms of viable cell counts (CFU/mL) using the standard plate method with ROGOSA agar after 48 h of incubation at 37°C. Serial dilutions of MJ were made in 1.0% (w/v) NSS before pour plating 1 ml onto the media.

The pH was measured with a Milwaukee pH 600 meter (Milwaukee Meters, USA). Total acidity, expressed as % lactic acid, was determined by titrating MJ with 0.1 N NaOH to pH 8.1. Experiments were performed at weekly intervals.

% lactic acid production was computed as:

$$\% \, lacticacid = \frac{\text{ml NaOH x N NaOH x 9.0}}{\text{wt. of sample (g)}}$$

Statistical analysis

All experiments were performed in triplicate and results are expressed as means and its standard errors (SEM). Paired t-tests and Repeated Measures Analysis of Variance (RMANOVA) were used to compare the mean of each lactobacillus across time. All the statistical tests used SPSS 17.0 and p-values of <0.05 indicate significant differences.

Results and Discussion

Survival under the simulated gastro-intestinal conditions

It is known from previous studies that, upon ingestion, probiotic microorganisms may come across several of human defence systems that are related to secretions. It is therefore essential to evaluate the sensitivity of the strains to be used to guarantee their effectiveness. Gastro-intestinal (GI) transit, which involves exposure to stomach acid, bile salts, and enzymes, is a major impediment for any particular candidate probiotic given that it must reach the intestine in viable form. Thus, acid and bile tolerances are among the criteria for selection of probiotic strains [18].

The survival of *Lactobacillus casei* BD II and *Lactobacillus plantarum* WCFS1 in simulated human stomach pH were observed at pH 1.0 and 3.0 for 0, 1, and 3 h (Table 1).The results showed that low pH had evident inhibition or killing effect on *L. casei* BD II and *L. plantarum* WCFS1. Initial counts prior to exposure ranged from 7.8 to 9.5 log cfu ml^{-1}. Both strains showed tolerance to pH 3.0 and residual counts were found to be 5.3 and 8.0 log values after 3 h exposure. *L. plantarum* survived significantly better than *L. casei* having only decreased by 15.6% after 3 h of exposure at pH 3.0, whereas the latter decreased by 32.7%. Furthermore, only *L. plantarum* remained viable after exposure to pH 1.0. The survival of *L. plantarum* at pH 1.0 significantly decreased from 5.7 to 5.1 log values (40.2% to 46.5%) after 1 h and was no longer detected after 3 h. In general, *L. plantarum* WCFS1 survived significantly better in the simulated stomach pH than *L. casei* BD II from 0 to 3 h, indicating that *L. plantarum* WCFS1 is more acid tolerant than *L. casei* BD II.

Table 1. Resistance of test isolates in low pH environment.

	Viability (log CFU/ ml) after treatment					
	pH 3.0			pH 1.0		
	0 h	1 h	3 h	0 h	1 h	3 h
***L. casei* BD II**	7.09 ± 0.01^a	6.88 ± 0.01^a	5.28 ± 0.03^a	ND	ND	ND
***L. plantarum* WCFS1**	9.01 ± 0.01^b	8.81 ± 0.01^b	8.02 ± 0.01^b	5.69 ± 0.02^c	5.09 ± 0.01^c	ND

Means and standard deviations for n = 3. The experimental values within columns that have no common superscript are significantly different (p<0.001) according to Duncan's multiple test range.
ND – not detected (<10^6 cfu ml^{-1})

Given that *Lactobacilli* are known to be tolerant at pH 4.6; the optimum pH of *L. casei* is 5.5, whereas *L. plantarum* is said to be more versatile and can grow at pH levels of ≥ 3.2. In this study, lower pH human stomach has a pH ranging from 1.0 to 4.5, the prior occurring during fasting and the latter occurring after a meal and complete digestion typically takes up to 3 h to complete [19]. *L. casei* BD II and *L. plantarum* WCFS1 showed better tolerance in pH 3.0 than pH 1.0 from 0 to 3 h. Results were in accordance with previous studies [15, 16, 18, 19] where *Lactobacilli* remained viable after exposure to pH values ranging from 2.5 to 4.0, but displayed loss of viability at lower pH values.

In a study conducted by Jacobsen *et al.*, [20], out of 44 *Lactobacillus* strains tested, none could replicate at pH 2.5. Berrada *et al.*, [22] mentioned that the tolerance of *L. casei* to acid was highly strain specific and only exhibited fairly good acid tolerance and survived pH 2.0 and/or pH 3.0, which could explain why *L. casei* BD II did not survive at pH 1.0, even during 0 h of exposure unlike *L. plantarum* WCFS1. Furthermore, according to Daeniel *et al.,* [23] it has been observed that when comparing different strains by means of *in vitro* experiments, the *L. casei* group of species appears to be the most sensitive. Thus, *L. casei* BD II may need protective adjustments like encapsulation prior to inoculation to be able to tolerate the low pH of the human stomach.

The survival of *L. casei* BD II and *L. plantarum* WCFS1 in the presence of digestive enzyme pepsin at pH 2.0 for 0, 1 and 3 h and pancreatin at pH 8.0 from 0 to 4 h is shown in Table 2. Initial counts prior to exposure at pH 2.0 and pH 8.0 ranged from 8.9 to 9.2 log cfu ml^{-1}. Based on the results, *L. plantarum* maintained its viability in the pepsin-pH solution after 3 h of exposure (5.1 log cfu ml^{-1}), contrary to *L. casei,* which lost its viability only after 1 h of exposure at pH 2.0. At 0 h, the survival of *L. plantarum* was significantly higher by having only decreased by 4.8%, whereas *L. casei* decreased by 48.5%. Further exposure of *L. plantarum* in thepepsin-pH solution significantly decreased from 4.8 to 45.0% after 3 h. In general, *L. plantarum* WCFS1 survived significantly better in the presence of pepsin than *L. casei* BD II from 0 to 3 h.

Table 2. Resistance of test isolates against digestive enzymes.

	Viability (log CFU/ml) after treatment				
	Pepsin pH 2.0			Pancreatin pH 8.0	
	0 h	1 h	3 h	0 h	4 h
***L. casei* BD II**	4.57 ± 0.13^a	ND	ND	7.73 ± 0.01	ND
***L. plantarum* WCFS1**	8.79 ± 0.01^b	5.14 ± 0.03^c	5.09 ± 0.02^c	8.37 ± 0.00^d	6.40 ± 0.03^e

Means and standard deviations for *n* = 3. The experimental values within columns that have no common superscript are significantly different (p<0.001) according to Duncan's multiple test range.
ND – not detected (<10^6 cfu ml^{-1})

Similarly, results show that *L. plantarum* WCFS1 survived significantly better in the presence of pancreatin enzymes than *L. casei* BD II from 0 to 4 h (Table 2). At 0 h, the survival of *L. plantarum* was significantly higher than that of *L. casei*, having 8.4 log cfu $ml^{-1}$and 7.7 log cfu ml^{-1}, respectively. After 4 h of exposure in pancreatin- pH solution, *L. casei* can no longer be detected, while *L. plantarum* significantly decreased from 9.4 to 31.0% having a final log value of 6.4.

Aside from having a low acidic environment, the stomach also produces pepsin, a digestive enzyme responsible for breaking down protein. The combined effect of the pepsin-pH solution aimed to simulate the gastric juice produced during digestion. An average person secretes about 400 ml of gastric juice per meal containing 50 to 300 µg ml^{-1} pepsin or 80 mg pepsin per meal [24]. The decrease in viability of *Lactobacillus* strains at pH 2.0 in the presence of pepsin was similar to the findings of [16, 25, 26]. According to previous studies, it is still unknown whether the decline in the number of viable cells was due to the pepsin alone, or in synergy with low pH. Meanwhile, in the small intestines where most enzymatic digestion occurs, pancreatic enzymes such as amylase, lipase and trypsin are being secreted to aid digestion [24]. The survival of *L. plantarum* WCFS1 in the presence of pancreatic enzymes up to 4 h indicates that it can survive the harsh environment in the small intestines, better than *L. casei* BD II.

As seen in Table 3, *L. casei* BD II and *L. plantarum* WCFS1 showed positive growth in the modified medium after 48 h of incubation at 37°C, which indicates that the test isolates exhibited good bile tolerance [17]. As mentioned earlier, probiotic microorganisms taken orally must withstand the presence of bile salts found in the GI tract. Bile tolerance is considered to be a key property for high survival and as a result of probiotic activity. The physiological concentration of bile salts in the small intestine is said to be between 0.2 and 2.0% [27]. The positive growth of *L. casei* and *L. plantarum* in the presence of 1.0% ox gall powder imply that the strains could survive the environment of the small intestine and reach the colon.

Table 3. Bile tolerance of test isolates.

	Growth after 48 h*
***L. casei* BD II**	(+)
***L. plantarum* WCFS1**	(+)

***(+) indicates growth in modified media containing 1.0% ox gall powder (w/v).**

According to Maragkoudakis *et al.,* [25] the *in vitro* screening of the survival of *Lactobacilli* in simulated GI tract conditions can only be considered in predicting the actual *in vivo* survival of a strain consumed in a non-protected way. Currently, very little is known or no information is available on the *in vivo* behaviour of ingested bacteria in the GI tract. It has been observed by other researchers that strains of *Lactobacilli* with established ability to colonize the human gut, still scored weakly when challenged *in vitro* [20, 23, 28].

Recent studies in developing probiotic products proposed that strains embedded in a food matrix may behave in a different way. The food matrix in particular, may have an effect on the survival of the microorganism during GI transit, such that it affects viability. The presence of fermentable sugars (e.g. glucose) in the acidic environment can help maintain the viability of some species of *Lactobacilli*. Glucose plays a significant role in the pH homeostasis of lactic acid bacteria. Studies show that under acidic conditions, the intracellular pH was higher in cells in medium containing glucose at lower pH environment. Glucose can enhance probiotic survival by providing the ATP pool required via glycolysis, permitting optimal proton extrusion by F_0F_1- ATPase (proton-translocating ATPase). Such mechanism can provide better delivery of viable probiotic *Lactobacilli* in the human GI transit. In addition, at least 8.33 g/L of glucose has a protective effect on *Lactobacilli* [29, 30].

Table 4. Survival ability of *L. casei* BD II and *L. plantarum* WCFS1 in mango juice during storage.

	week 0	week 1	week 2	week 3	week 4
Storage: 4°C					
***L. casei* BD II**[a]	8.37 ± 0.02	8.28 ± 0.00	7.84 ± 0.00	6.95 ± 0.05	ND
***L. plantarum* WCFS1**[b]	8.38 ± 0.04	8.28 ± 0.02	7.94 ± 0.04	6.9 ± 0.05	6.59 ± 0.14
Storage: 25 ± 2°C					
***L. casei* BD II**[c]	TNTC	8.09 ± 0.01	7.94 ± 0.03	7.56 ± 0.03	ND
***L. plantarum* WCFS1**[d]	TNTC	8.29 ± 0.01	7.64 ± 0.08	7.55 ± 0.10	ND

Means and standard deviations for $n = 3$. The test isolates that have no common superscript are significantly different ($p < 0.05$) according to Duncan's multiple test range.
TNTC- too numerous to count (>10^8 cfu ml^{-1})
ND - not detected (<10^6 cfu ml^{-1})

In this context, even strains not able to survive at pH 1.0 *in vitro* could still display considerable viability when consumed as adjuncts in a food matrix that is high in glucose and contains prebiotic pectic oligosaccharides (POS), such as mango juice. Therefore, *L. casei BD* II despite scoring poorly in the *in vitro* screening was still inoculated in the formulated mango juice.

Viability of lactobacillus strains in mango juice

The viability of lactobacillus strains in mango juice (MJ) is shown in Table 4. MJ was rapidly utilized by both species of lactic acid bacteria, reaching a viable cell population of ≥1.0x10^8cfu ml^{-1} after 48 h of fermentation (week 0), with an excess of ≥2 log cycles from the initial inocula (~10^6 cfu ml^{-1}). Storage of MJ at higher temperature (25 ± 2°C) significantly increased the fermentation process compared to low temperature (4°C). Viable cells of *L. casei* and *L. plantarum* in MJ stored at 4°C ranged from 8.37 ± 0.02 to 8.38 ± 0.04 log cfu ml^{-1}at week 0. Conversely, the initial viability of *L. casei* and *L. plantarum* in MJ stored at 25 ± 2°C reached ≥1.10^{10}cfu ml^{-1}. Viability of *L. casei* and *L. plantarum* in MJ stored at 4°C and 25 ± 2°C significantly decreased from 0 to 4 weeks. Notably, viable cell counts in the MJ stored at 25 ± 2°C significantly decreased by ~3 log cycles between week 0 and week 4 to levels below the critical value of 10^6cfu ml^{-1}. By week 3, the viability of *L. casei* and *L. plantarum* at 4°C were both significantly less than at 25 ± 2°C. Furthermore, *L. casei* at 4°C decreased its viability 16.92% after 3 weeks and could no longer be recovered at week 4. On the other hand, *L. plantarum* stored at 4°C decreased its viability from 17.6 to 21.4%, after week 3 and week 4.

Change of pH and acidity in mango juice containing probiotic strains

Changes in total acidity expressed as % lactic acid and change in pH of MJ are shown in Figures 1 and 2. The total acidity of MJ stored at 4°C and 25 ± 2°C have significant differences ($p<0.05$) and showed increasing acidity from 0 to 4 weeks. Post hoc analysis indicated that MJ containing *L. casei* and *L. plantarum* stored at 4°C significantly produced the least change in titratable acidity ranging from 0.26 to 0.29% from the initial 0.21% titratable acidity. In addition, MJ containing *L. plantarum* stored at 25 ± 2°C produced significantly more titratable acidity (1.18%) compared to MJ containing *L. casei* stored at 25 ± 2°C (0.52%). Generally, all treatments containing lactic acid cultures had a decreasing change in pH from 0 to 4 weeks of storage, contrary to untreated MJ samples, which maintained their pH of 3.9 (data not shown). The most notable changes in pH were evident in MJ stored at 25 ± 2°C. By week 4, *L. plantarum* decreased its pH to 2.8, while *L. casei* decreased its pH to 3.3. The least changes in pH of MJ were detected from those stored at 4°C; *L. casei* decreased its pH to 3.8 and *L. plantarum* decreased its pH to 3.7 after 4 weeks of storage.

The prospective use of fruit-based matrices as carriers of probiotic microorganisms is limited by the fact that fruit juices can inhibit the growth of lactic acid bacteria (strain-specific effect) due to its naturally low pH. Factors affecting cell viability also include the following: interaction between species present, culture condition, oxygen content, final acidity of the product and the concentration of lactic acid and acetic acid [1] and [31]. However, the primary reason for the decline in viability of lactic cultures have been attributed to the decrease in pH of the medium and build-up of organic acids resulting from growth and fermentation [32, 33, 34].

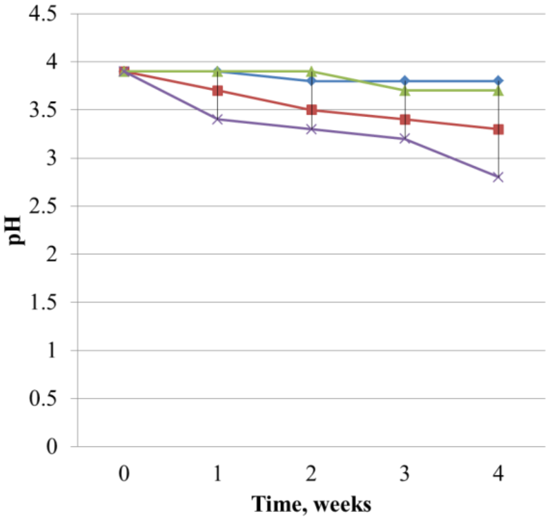

Figure 1. Change of pH in MJ containing *L. casei* stored in 4°C (◇) and in 25°C (□), *L. plantarum* stored in 4°C (Δ) and in 25°C (x).

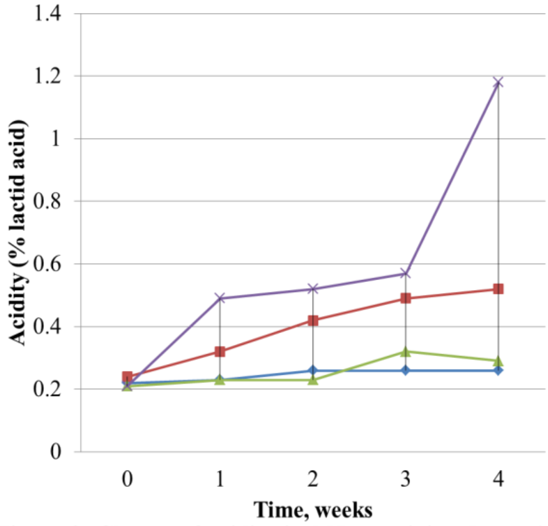

Figure 2. Change of acidity in MJ containing *L. casei* stored in 4°C (◇) and in 25°C (□), *L. plantarum* stored in 4°C (Δ) and in 25°C (x).

In this study, *L. casei* BD II and *L. plantarum* WCFS1 survived the low pH of MJ and easily utilized its natural carbohydrates for growth and fermentation. Based on the results, higher temperature storage (25 ± 2°C), accelerated chemical and enzymatic reactions of *Lactobacilli* in the medium resulting in the production of inhibitory substances, while the use of refrigeration (4°C) impeded metabolic activities and resulted in better survival during prolonged storage.

Conclusions and Recommendations

Novel probiotic strains *L. casei* BD II and *L. plantarum* WCFS1 possess technological properties that are suitable for food applications. Mango juice may also serve as a raw material for lactic acid fermentation and the product could be a refreshing alternative healthy beverage. Based on the findings of this study, microencapsulation of *L. casei* BD II and *L. plantarum* WCFS1 is highly recommended to enhance the viability of cells when inoculated into fruit juice. It is also highly recommended that future *in vivo* studies on the efficacy of the probiotic mango juice be performed to further establish that mango is an ideal substrate for probiotic microorganisms.

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