Food Chemistry 139 (2013) 261–266

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Sonicated pineapple juice as substrate for *L. casei* cultivation for probiotic beverage development: Process optimisation and product stability

Mayra Garcia Maia Costa, Thatyane Vidal Fonteles, Ana Laura Tibério de Jesus, Sueli Rodrigues *

Departamento de Tecnologia de Alimentos, Universidade Federal do Ceará, Campus do Pici Bloco 858, Caixa Postal 12168, CEP 6002-1970 Fortaleza, Ceará, Brazil

ARTICLE INFO

Article history:
Received 1 September 2012
Received in revised form 16 January 2013
Accepted 21 January 2013
Available online 1 February 2013

Keywords:
Probiotic
Pineapple juice
Lactobacillus casei

ABSTRACT

The aim of this study was to evaluate the use of sonicated pineapple juice as substrate for producing a probiotic beverage by *Lactobacillus casei* NRRL B442. Maximal microbial viability was found by cultivating *L. casei* at 31 °C and pH 5.8 (optimised conditions). After fermentation, samples of sweetened and non-sweetened juice were stored. After 42 days of storage under refrigeration (4 °C), the microbial viability was 6.03 Log CFU/mL in the non-sweetened sample and 4.77 Log CFU/mL in the sweetened sample. The pH of both samples decreased during storage due to lactic acid production (post acidification). The characteristic colour of the juice was maintained throughout the shelf life and no browning was observed. Sonicated pineapple juice was shown to be a suitable substrate for *L. casei* cultivation and for the development of an alternative non-dairy probiotic beverage.

© 2013 Elsevier Ltd. Open access under the Elsevier OA license.

1. Introduction

To date, emerging and innovative technologies such as radiation processing, hydrothermal treatments, osmotic dehydration, pulsed electric field applications and others have been explored in food processing to improve shelf life and to preserve nutritional and organoleptic qualities of fresh fruits or their products. Sonication (ultrasound) treatment, which is an emerging technology that is considered to be inexpensive, simple, reliable and environmentally friendly, has been studied for use in several applications including fruit juice processing (Bhat, Ameran, Karim, & Liong, 2011; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2009; Valero et al., 2007). According to O'Donnell, Tiwari, Bourke, & Cullen (2010), ulltrasonic processing of fruit juices has minimal effects on the quality of fruit juices such as orange juice (Valero et al., 2007), guava juice (Cheng, Soh, Liew, & Teh, 2007) and strawberry juice (Tiwari, O'Donnell, Patras, & Cullen, 2008).

The regular consumption of probiotic microorganisms is associated with bowel function regulation, improvement of lactose digestion, stimulation of the immune system and the inhibition of pathogens. By definition, probiotics need to be viable at the time of consumption, although non-viable "probiotics" are not necessarily without health effects (Ouwehand & Salminen, 1998). As a general rule, a lower limit of 10^9 colony forming units (CFU) per dose is often used, although this may be different depending on the strain, health effect and possibly even the matrix (Forssten, Sindelar, & Ouwehand, 2011).

Probiotics, in particular *Lactobacillus* and *Bifidobacterium*, are commercially found in fermented milk and yoghurt. Due to their physiology, they are very well suited to these kinds of food matrices. However, recent studies have suggested fruit juices as an alternative vehicle for the incorporation of probiotics (Fonteles, Costa, de Jesus, & Rodrigues, 2011; Mousavi, Mousavi, Razavi, Emam-Djomeh, & Kiani, 2011; Pereira, Maciel, & Rodrigues, 2011; Sheehan, Ross, & Fitzgerald, 2007; Yoon, Woodams, & Hang, 2006). Fruit juices are rich in nutrients and do not contain starter cultures that compete for nutrients with probiotics. Furthermore, fruit juices are often supplemented with oxygen scavenging ingredients such as ascorbic acid, thus promoting anaerobic conditions. Fruit juices contain high amounts of sugars, which could encourage probiotic growth and could easily be monitored using a refractometer (Ding & Shah, 2008).

Lactobacilli are extensively used in industrial food production and, today, as functional ingredients (Havenaar, Brink, & Veld, 1992). The growth of lactobacilli is affected by fermentation conditions, such as pH, temperature, media formulation and others (Liew, Ariff, Raha, & Ho, 2005). According to Ranadheera, Baines, and Adams (2010) the food vehicle can also influence parameters of probiotic growth, survival and functionality. The genus *Lactobacillus* can grow in the pH range from mild acid to neutral values and temperatures from 2 to 53 °C, with optimum temperature range of 30–40 °C and optimum pH range of 5.5–6.2 (Dicks & Endo, 2009). However, due to the bacterial individual characteristics of growth rate, metabolism and proteolytic activity, successful addition of probiotics to food depends on the species, strain, possible interactions with other bacteria and the pH of the food matrix. The presence of oxygen and the temperatures of fermentation and storage

* Corresponding author. Tel.: +55 85 3366 9656; fax: +55 85 3458 3407.
E-mail address: sueli@ufc.br (S. Rodrigues).

0308-8146 © 2013 Elsevier Ltd. Open access under the Elsevier OA license.
http://dx.doi.org/10.1016/j.foodchem.2013.01.059

also affect microbial viability (Ferreira et al., 2005; Vinderola & Reinheimer, 2000). The optimum range of microbial development is dependent on the physical and chemical parameters of the substrate and, to evaluate the growth of lactic acid bacteria, it is necessary to know the substrates applied for the microbial growth, as well as the optimal temperature and pH values because these factors are the most important for microbial development (Du Toit, Engelbrecht, Lerm, & Krieger-Weber, 2011).

Fruit juices have an established market sector as functional drinks through sales of juices fortified with calcium and vitamins and they are consumed regularly, which is essential if the full benefits attributed to probiotics are to be experienced (Sheehan et al., 2007). Pineapple juice sonication was previously studied by Costa et al. (2011). According to the authors, juice sonication reduced the polyphenoloxidase (PPO) activity by 20% and the juice viscosity by 75%. Sonicated fruit juices can be applied for several uses including the development of ready to drink beverages.

To date the use of sonicated fruit juices as substrate for probiotic microorganisms has not been evaluated. Due to positive results reported on fruit juice sonication due to its low cost and high efficiency as a technology for fruit juice processing, the aim of the present study was to evaluate the hypothesis that sonication could be applied in fruit juices prior to fermentation. Thus, the use of sonicated pineapple juice as substrate to produce a probiotic fruit juice was studied herein.

2. Methods

2.1. Pineapple juice

Fresh natural pineapple pulp (*Ananas comosus* L., Perola variety) was purchased from the local market. The juice was prepared by dissolving 100 g of pulp in 100 mL of potable water and the mixture was then homogenised by sonication at 376 W cm^{-2} for 10 min in a 500 W ultrasonic processor (Unique® DES500, São Paulo, Brazil) with a 1.3 cm probe tip. Samples were processed at a constant ultrasonic frequency of 19 kHz.

2.2. Microorganism and inoculum preparation

A strain of *Lactobacillus casei* NRRL B-442 obtained from ARS Culture Bacterial Collection (NRRL Culture collection, United States Department of Agriculture, Peoria, IL, USA) was statically activated for 12 h at 37 °C in 250 mL Erlenmeyer flasks containing 100 mL of MRS broth (de Man, Rogosa, & Sharpe, 1960). The initial pH of the culture medium was adjusted to 6.5 with H_3PO_4. From this culture, stock cultures were prepared by adding sterile glycerol (50% v/v) to the activated culture. The glycerol stock culture was stored frozen (−20 °C) in sterile screw caps tubes containing 8 mL of the culture suspension.

Inoculums were prepared by transferring a glycerol stock culture tube of *L. casei* B-442 to a 250 mL Erlenmeyer flask containing 100 mL of sterile MRS broth. Cell cultivation was carried out statically in an incubator at 37 °C until the cell density, spectrophotometrically determined at 590 nm, reached 0.600, which corresponds to 9.00 Log CFU/mL according to the MacFarland scale (Fonteles et al., 2011). This culture was used as inoculum to the juice fermentation.

2.3. Pineapple juice fermentation

The optimum fermentation conditions were determined by response surface methodology (RSM). A central composite rotated experimental design (CCRD) was carried out. The initial pH and temperature ranged from 4.29 to 7.11 and 10.44 to 41.44 °C (Table 1). The experimental domain was chosen based on the range that *Lactobacillus* strains can grow: pH from mild acid to neutral values and temperature from 2 to 53 °C. Initial pH values of all experimental runs were adjusted to reach the desired values (Table 1) with NaOH (120 g/L). According to the Brazilian legislation, NaOH can be used as a food additive for use as an acidity regulator (Anvisa, 2007; Pereira et al., 2011).

Two millilitres of the inoculum, containing 9.0 CFU/mL of *L. casei*, were added to 500 mL Erlenmeyer's flasks containing 200 mL of pineapple juice. Thus, the initial cell count in the juice was 7.0 Log CFU/mL. Fermentation was carried out statically in an incubator for 24 h at the different temperatures of the experimental design (Table 1). Biomass and viable cell counts were determined at the end of the process.

2.4. Microbial growth and viable cell count determination

The growth of *L. casei* was quantified by measuring the optical density at 590 nm. The absorbency was recorded for the fresh juice inoculated with *L. casei* (initial absorbance) and after 24 h of fermentation (final absorbance). The procedure consisted of diluting with distiled water an aliquot of the juice containing the microbial cells and reading the absorbance at 590 nm against water. The difference between final and initial absorbance corresponded to the growth of the microorganisms during the fermentation. Growth was expressed as dry mass concentration (g/L) calculated using the calibration curve given in Eq. (1), built using *L. casei* dry cells.

$$L.\ casei(\mathrm{g/L}) = \frac{\mathrm{ABS}(590\ \mathrm{nm}) - .008}{3.395} \quad (1)$$

Serial dilutions of fermented pineapple juice in sterile peptone water up to 10^{-7} were done for viable cell counts. Aliquots of 0.1 mL of the diluted fermented juice were inoculated on plates containing MRS agar, plating on the surface with the aid of a handle Drigalsky. Samples were seeded in triplicate. The plates were incubated inverted at 37 °C for 72 h. Typical colonies of *L. casei* were counted. *L. casei* colonies are round white creamy, with diameters ranging from 0.9 to 1.3 mm (Vinderola & Reinheimer, 2000). The pineapple juice pH was determined by direct measure in a Marconi PA 200 potentiometer.

2.5. Kinetic study

A kinetic study was carried at the optimum fermentation conditions (pH 5.8 and 31 °C) determined by response surface methodology to determine the proper fermentation time for pineapple juice fermentation.

2.6. Stability assay

Pineapple juice was fermented at the optimum conditions for 12 h, as determined in the kinetic study. After fermentation, the juice was separated into two portions: to one portion, 10% (w/v) of sugar was added and to the other, no sugar was added. The samples were bottled in 200 mL screw caps transparent glass bottles containing 150 mL of juice each and stored under refrigeration (4 °C) for 42 days. Each 7 seven days, a bottle of each sample (sweetened and non-sweetened) was analysed (pH, microbial viability and colour). A control sample (non-fermented juice) containing sodium azide (1% w/v) was also evaluated throughout the storage stability study.

2.7. Sugars and lactic acid determination

The cells were harvested by centrifugation at 11.806*g* for 10 min in a Sigma 6K-15 centrifuge. The supernatant containing

Table 1
Experimental design and responses on *Lactobacillus casei* NRRL B-442 growth and viability in pineapple juice after 24 h of fermentation.

Assay	Initial pH	Fermentation temperature (°C)	Growth (g/L)	Viable cell counts (Log CFU/mL)
1	4.70 (−1)	15.00 (−1)	0.30 ± 0,12	6.34 ± 0.00
2	4.70 (−1)	37.00 (+1)	1.23 ± 0.19	8.34 ± 0.10
3	6.70 (+1)	15.00 (−1)	0.18 ± 0.08	6.64 ± 0.03
4	6.70 (+1)	37.00 (+1)	0.94 ± 0.03	8.04 ± 0.27
5	4.29 (−α)	26.00 (0)	0.91 ± 0.19	8.29 ± 0.15
6	7.11 (+α)	26.00 (0)	0.89 ± 0.06	8.87 ± 0.03
7	5.70 (0)	10.44 (−α)	0.06 ± 0.05	6.66 ± 0.01
8	5.70 (0)	41.44 (+α)	1.02 ± 0.08	8.51 ± 0.06
9	5.70 (0)	26.00 (0)	0.97 ± 0.02	8.98 ± 0.02
10	5.70 (0)	26.00 (0)	1.07 ± 0.01	8.67 ± 0.07
11	5.70 (0)	26.00 (0)	1.05 ± 0.11	8.59 ± 0.18

the sugars and lactic acid was analysed by high performance liquid chromatography in a Varian ProStar system equipped with two high-pressure pumps (ProStar Model 210), refractive index detector (ProStar 355 RI), UV–VIS detector (ProStar Model 345) and column oven (Timberline). Separation was done using an Aminex HPX 87H (300 × 7.8 mm) column at 50 °C. Sulphuric acid (0.02 M) in ultrapure water at 0.6 mL/min was used as eluent, and the RI detector temperature was set at 35 °C. Lactic acid was detected at 210 nm. All samples were analysed in triplicate. ProStar WS 5.5 software was used to acquire and handle the data.

2.8. Colour

The colour of the fermented pineapple juice was determined using a Minolta CR300 colorimeter (Tokyo, Japan). The colorimeter was calibrated using the illuminant D65, and measurements were made through an 8-mm port/viewing area (Minolta, 1998). The reflectance instruments determined three colour parameters: lightness (L^*), redness (a^*), and yellowness (b^*). Numerical values of L^*, a^* and b^* were converted into ΔE^* (total colour difference), ΔC (chroma) and *hue* angle ($h°$) according to Eqs. (2)–(4), respectively. colour measurements were taken in quintuplicate.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{2}$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \tag{3}$$

$$h^\circ = \tan^{-1}(b^*/a^*) \tag{4}$$

2.9. Sensory evaluation

Sensory evaluation was carried out with 20 non trained panelists. Sweetened and non-sweetened probiotic pineapple juice was served cold (4 °C) in a transparent glass. Panelists were asked to choose between the two samples (sweetened and non-sweetened) and to rate the juice colour as dark or acceptable for a pineapple juice based beverage. The sensory test was carried out after 21 days of cold storage for both samples.

2.10. Statistical analysis

Statistica software version 7.0 (Statsoft, USA) was used to build the experimental design, the surface graphs and to analyse the results. The results obtained were presented as mean values with standard deviation.

3. Results and discussion

The results of the experimental design are presented in Table 1. According to the results, assays 1, 3 and 7 presented a discrete decrease in viable cell count of *L. casei* (initial cell counts were 7 Log CFU/mL). This behaviour confirms the mesophilic characteristic of the studied strain because low temperatures were not favourable for *L. casei* B-442 cultivation. For all other assays, an increase of at least 1 Log CFU/mL was observed. According to the estimated effects of the independent variables on the studied responses, initial pH was not a significant influence on cell growth and viability ($p < 0.05$). However, *L. casei* growth and viability were significantly influenced by temperature. The fitted models obtained for the data presented in Table 1 are given in Eqs. (5) and (6).

$$\text{Growth(g/L)} = -4.16 + 0.95\text{pH} - 0.08\text{pH}^2 + 0.16T - 0.002T^2 - 0.003\text{pH} * T \tag{5}$$

$$\text{Viability(Log UFC/mL)} = -8.6 + 3.54\text{pH} - 0.27\text{pH}^2 + 0.47T - 0.006T^2 - 0.01\text{pH} * T \tag{6}$$

where pH initial pH; T temperature of fermentation (°C).

The fitted models were validated by ANOVA analysis and *F*-test. Both models were statistically significant because the calculated F value (34.44 for growth and 6.10 for viability) were greater than the listed F value ($F_{5,5}$ = 5.05) at 95% confidence level. The determination coefficient (R^2) was 0.95 for both fitted models. Fig. 1a and b are the surface graphs built using Eqs. (5) and (6), respectively. At low temperatures, a discrete growth of *L. casei* was observed. However, as the temperature increased microbial growth also increased reaching a maximum in the pH range from 5.5 to 6.0 and temperature range from 30 to 40 °C. The optimum operating conditions for microbial growth (critical point of Eq. (2)) were pH 5.1 and initial fermentation temperature 34.5 °C. The critical point is the zero derivative of the equation (maximal point) and was calculated using the Statistica software.

Similar behaviour was observed for cell viability (Fig. 1b). As the fermentation temperature increased up to 31 °C, cell viability increased. At higher temperatures, cell viability decreased, despite the good growth observed in Fig. 1a. The optimal operating conditions for cell viability (critical point of Eq. (6)) were obtained at pH 5.8 and 31 °C.

The optimum operating condition was different for microbial growth and cell viability. Pereira et al. (2011), studying the fermentation of cashew apple juice with *L. casei*, reported the highest viability at pH 6.4 and fermentation temperature of 30 °C. The optimum microbial growth was reported at 35 °C. Fonteles et al. (2011) reported optimum fermentation conditions for probiotic cantaloupe melon juice fermentation at 31 °C and initial pH of 6.1, good growth and viability of probiotic microorganisms occurred. Pineapple juice presented different optimal conditions compared to cashew and melon juices because the food matrix affects microbial growth. *Lactobacillus* strains are known as nutritional exigent microorganisms. In a poor substrate the strain is usually unable to grow. Thus, sonication cannot have caused significant nutrient losses in pineapple juice for, if it had, *L. casei* would not have been able to grow in the juice.

The optimum processing conditions for microbial viability (initial pH 5.8 and 31 °C) were chosen in which to evaluate juice fermentation over 24 h. Fig. 2 presents the microbial viability (Log CFU/mL) and the juice pH during the course of fermentation. Over 24 h of fermentation, a sharp increase of microbial viability was observed from 8 to 10 h of fermentation, reaching 8.34 Log CFU/mL. No significant increase was observed thereafter up to 24 h of fermentation when the viability reached 8.65 Log CFU/mL.

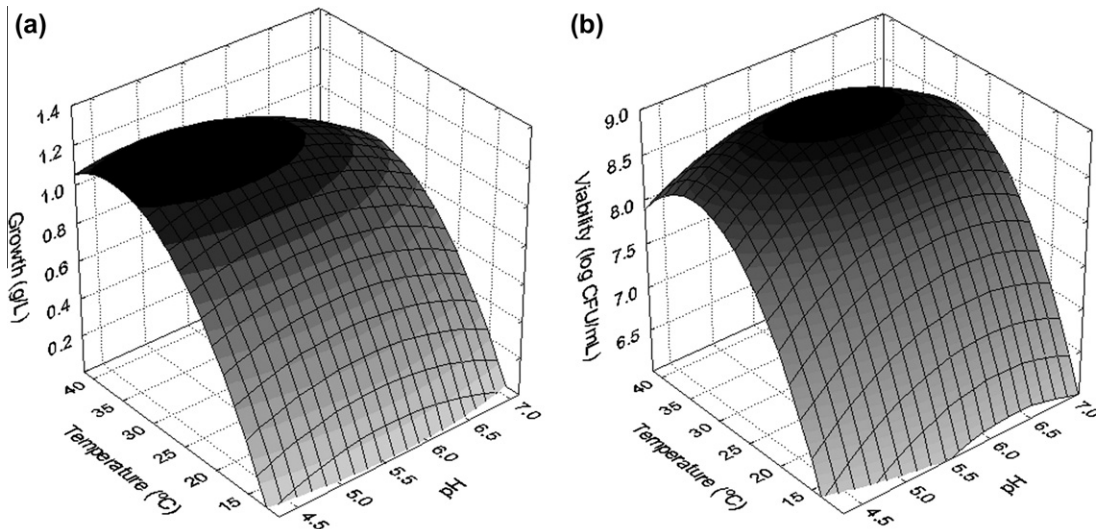

Fig. 1. Surface graph of growth (g/L) *Lactobacillus casei* NRRL B-442 in pineapple juice as function of initial pH and temperature (a); Surface graph of viability (Log CFU/mL) of *Lactobacillus casei* NRRL B-442 in pineapple juice as function of initial pH and temperature (b).

Stopping the process at 10 h could lead to a pH value too close to that of 4.5, which is the threshold considered for the development of many pathogens in food. On the other hand, allowing an additional 2 h of fermentation, the pH dropped to 4.21, which is safer for product storage (Fig. 2a). This pH decrease was due to lactic acid production because of the microbial growth, as seen in the 12 h fermentation window (Fig. 2b).

The results presented herein are in agreement with other studies (Gupta, Ghannam, & Scannell, 2010; Yoon et al., 2006), which suggested that different vegetable matrices could serve as good media for growing probiotics by stimulating their growth, resulting in good viable counts. Maximal growth was obtained at different conditions of cell viability. However, cell viability is the key factor for a functional product. Thus, the optimum conditions for cell viability were applied to the kinetic study. Usually, *Lactobacilli* strains are reported to present optimum growth at 37 °C and pH around 6.5. Nazarro, Fratianni, Sada, and Orlando (2008) evaluated the possibility of producing a functional vegetaable beverage based on the growth of *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* in carrot juice. Both bacterial strains were capable of growing in carrot juice, reaching nearly 9 Log CFU/mL after 48 h of fermentation, and the pH was reduced to 3.5–3.7 or below. As reported elsewhere, the results presented herein confirm that microbial survival in foods is strongly dependent on the food matrix (Shah, 2007).

Fig. 3 shows the carbohydrate consumption that occurred during the fermentation. The major sugar in pineapple juice was sucrose (~86%). Sucrose decreased during the fermentation while glucose and fructose increased. Carbohydrates are consumed during the fermentation due to the microbial growth. The pH decreased due to lactic acid production and sucrose hydrolysis occurred due to low pH values. From data presented in Fig. 3, the rate of sucrose hydrolysis was faster than that of sugar consumption, thus resulting in an increase of reducing sugars.

A newly fermented sample was prepared using the optimised conditions (initial pH of 5.8, 31 °C and 12 h of fermentation) for the storage stability assay. After 12 h of fermentation, the juice was divided into two sample categories: sweetened samples containing 10% w/v of table sugar (sucrose) and non-sweetened samples. The samples were bottled and stored as earlier described. Fig. 4a and b present details of the microbial viability and the pH throughout the storage period of the fermented sonicated pineapple juice (4 °C/42 days). Fig. 4c and d present data on sugar consumption in the samples. For both samples, pH and viability decreased during the storage period. A sharp drop in pH values was clearly observed for both samples during the first week of storage. This behaviour is consistent with high sugar consumption and indicates that post-acidification occurred at this period. Higher acidification and higher sugar consumption was observed for sweetened juice. Microbial viability was almost linear during the storage period exhibiting higher losses for sweetened sample due to lower pH values. Non-sweetened juice exhibited microbial viability higher than 6 Log CFU/mL for the whole storage period

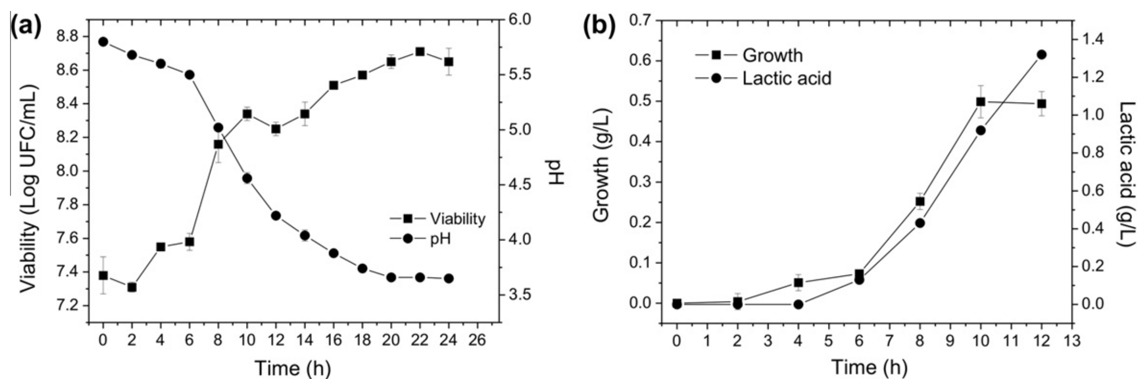

Fig. 2. Graph of growth, viability and pH of *L. casei* NRRL B-442 over 24 h of fermentation in pineapple juice.

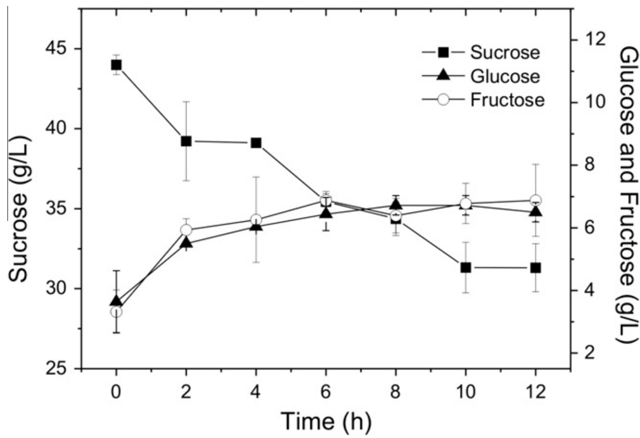

Fig. 3. Carbohydrate consumption during pineapple juice fermentation under optimised conditions (pH 5.8/31 °C).

(42 days). On the other hand, sweetened juice had a shorter shelf life because cell counts were maintained above 6 Log CFU/mL for 28 days. Sugar profile showed the same behaviour observed during the fermentation. Sucrose concentration decreased and glucose and fructose increased. Again sucrose hydrolysis rate was faster than the rate of sugar consumption and higher reducing sugar levels were obtained for both samples. In addition to higher acidity, the sweetened juice also had a higher osmotic pressure when compared to the non-sweetened juice, which might have contributed to the lower microbial viability of *L. casei* during the storage period. Pereira et al. (2011) studied the storage stability of cashew apple juice fermented with *L. casei* under the same conditions and reported that the microbial viability increased during the storage period, up to 28 days, decreasing thereafter. In probiotic cashew apple juice (non-sweetened), viable cell counts were higher than 8 Log CFU/mL during the 42 days of cold storage, attesting again to the strong effect of food matrix on microbial survival rates. Despite the probiotic sonicated pineapple juice presenting lower viable cells compared to other juices, a portion of 100 mL of the sweetened juice would reach the recommended ingestion for dairy products of 9 Log CFU if the juice were consumed within 21 days of cold storage. On the other hand, non-sweetened juice presented a longer shelf life (35 days under cold storage).

Colour analysis results of fermented and non-fermented sonicated pineapple juice are presented in Table 2. The hue angle ($h°$) showed variation of less than 5°, indicating that the characteristic colour of the juice (yellow) was maintained throughout the storage period for both samples (non-fermented and fermented). No significant browning was observed during juice storage. This fact is consistent with the values of a^* parameter that were kept at low negative values (within the yellow range in the colour wheel). Sonication promotes enzyme inactivation, which contributes to the characteristic colour maintenance. Non-fermented juice presented some colour changes during the storage period, as shown by the decrease of b^* and Chroma, accompanied by an increase of ΔE after 14 days of storage. The colour of the fermented juice presented a slight reduction in the yellow colour intensity indicated by the decrease of b^* and Chroma just after fermentation processing. However, this change did not cause a significant visual difference when compared to the non-fermented juice due to the low ΔE value. During the storage period, the colour difference between the fermented and non-fermented juice increased at 7 days of storage, decreasing after that up to the end of the fermented juice shelf life (28 days). These differences are attributed to lower L^* values obtained for the fermented juice compared to non-fermented juice. Thus, fermented pineapple juice presented a more saturated yellow colour compared to the non-fermented juice.

The sensory evaluation of the samples (sweetened and non-sweetened) revealed a greater than 75% preference for the

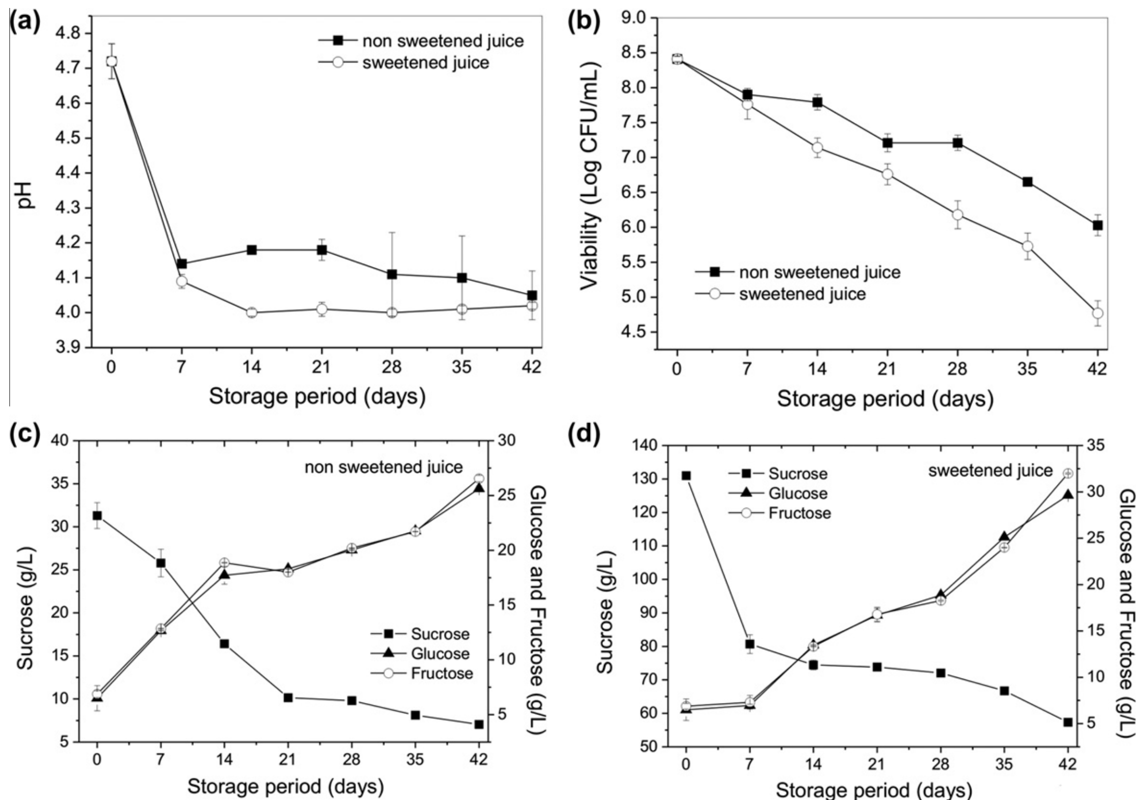

Fig. 4. Microbial viability (a); pH (b); sugar consumption: non sweetened juice (c) sweetened juice (d), during the storage period (4 °C/42 days).

Table 2
Colour parameters during cold storage of non-fermented (*in natura*) and fermented pineapple juice.

Storage period (days)	L^*	a^*	b^*	Chroma (C)	Hue ($h°$)	ΔE^*
Non-fermented juice	79.67 ± 0.17	−3.64 ± 0.02	17.03 ± 0.12	17.41	102	
7	79.80 ± 0.09	−3.19 ± 0.03	17.04 ± 0.41	17.34	101	0.54
14	76.12 ± 0.41	−2.86 ± 0.10	12.19 ± 0.79	12.52	101	5.95
21	76.05 ± 0.50	−2.79 ± 0.17	12.83 ± 0.79	13.13	102	5.50
28	76.22 ± 0.15	−2.95 ± 0.07	12.28 ± 0.31	12.63	104	5.81
Fermented juice*	81.73 ± 0.31	−2.67 ± 0.06	12.98 ± 0.28	13.25	102	2.37
7	71.53 ± 0.13	−3.15 ± 0.02	16.40 ± 0.17	16.69	101	6.31
14	73.02 ± 0.41	−2.78 ± 0.08	15.11 ± 0.61	15.36	100	4.26
21	73.15 ± 0.34	−2.46 ± 0.09	14.50 ± 0.55	14.70	101	3.37
28	72.12 ± 0.86	−2.80 ± 0.12	13.22 ± 1.14	13.51	100	4.21

* Chroma, hue and ΔE were calculated against the *non-fermented* juice.

sweetened sample. Colour was considered acceptable for a pineapple juice product by 90% of the consumers for both samples. The preference for the sweetened fermented juice might be attributed to the fact that juice sugars are consumed during the fermentation and storage for microbial growth. Sugar addition increased the sugar level compensating for the sugar depletion due to fermentation. In addition, lactic acid is produced during the storage (post acidification). The increase in sugar levels in sweetened juice also will have contributed to reducing the acidic sensation by increasing the ratio of Brix/acidity.

4. Conclusion

Despite the great potential for the use of fruit juice as probiotic carriers, little work has been done in this field to consider fermented juices. Most reported studies are based on microbial addition of probiotic strains to fruit juices. This study showed that sonication can be applied as a pre-treatment for cultivating the probiotic strain *L. casei* B-442, which was then able ferment sonicated pineapple juice without any nutrient supplementation. Good viable cells counts were obtained in a short time (12 h) and microbial viability was maintained within the acceptable range for at least 21 days under cold storage. Browning, which is characteristic of pineapple juice and usually is avoided using chemical products such as sodium metabisulfite, was prevented only by applying sonication before fermentation. The juice colour was well accepted and consumers indicated a preference for the sweetened product. Complementary studies on the impact of the fermentation process on sensory acceptance as well as the use of non-caloric sweeteners such as stevia and sucralose are to be the subject of future studies.

Acknowledgments

Authors thank CNPq for the financial support through the Nacional Institute of Science and Technology of Tropical Fruit and CAPES for the scholarship.

References

Anvisa. *National Agency of Sanitary Vigilance through Governmental*. Informe Técnico n°33, de 25 de outubro de 2007. Hidróxido de sódio – INS 524, Brasília. 2007.

Bhat, R., Ameran, S. B., Karim, A. A., & Liong, M. T. (2011). Quality attributes of starfruit (*Averrhoa carambola* L.) juice treated with ultraviolet radiation. *Food Chemistry, 127*, 641–644.

Cheng, L. H., Soh, C. Y., Liew, S. C., & Teh, F. F. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry, 104*, 1396–1401.

Costa, M. G. M., Fonteles, T. V., Jesus, A. L. T., Almeida, F. D. L., Miranda, M. R. A., Fernandes, F. A. N., et al. (2011). High-Intensity ultrasound processing of pineapple juice. *Food and Bioprocess Technology*. http://dx.doi.org/10.1007/s11947-011-0746-9.

de Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of *Lactobacilli*. *Journal of Applied Bacteriology, 23*, 130–135.

Dicks, L. M. T., & Endo, A. (2009). Taxonomic status of lactic acid bacteria in wine and key characteristics to differentiate species. *South African Journal for Enology and Viticulture, 30*, 72–90.

Ding, W. K., & Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *International Food Research Journal, 15*, 219–232.

Du Toit, M., Engelbrecht, L., Lerm, E., & Krieger-Weber, S. (2011). *Lactobacillus*: The next generation of malolactic fermentation starter cultures—An overview. *Food Bioprocess and Technology, 4*, 876–906.

Ferreira, V., Soares, V., Santos, C., Silva, J., Gibbs, P. A., & Teixeira, P. (2005). Survival of *Lactobacillus sakei* during heating, drying and storage in the dried state when growth has occurred in the presence of sucrose or monosodium glutamate. *Biotechnology Letters, 27*, 249–252.

Fonteles, T. V., Costa, M. G., de Jesus, A. L. T., & Rodrigues, S. (2011). Optimization of the fermentation of cantaloupe juice by *Lactobacillus casei* NRRL B-442. *Food and Bioprocess Technology*. http://dx.doi.org/10.1007/s11947-011-0600-0.

Forssten, S. D., Sindelar, C. W., & Ouwehand, A. C. (2011). Probiotics from an industrial perspective. *Anaerobe, 6*, 410–413.

Gupta, S., Ghannam, N. A., & Scannell, A. G. M. (2010). Growth and kinectics of *Lactobacillus plantarum* in the fermentation of edible Irish brown seaweeds. *Food Bioproducts and Processing, 89*, 346–355.

Havenaar, R., Brink, B. T., & Huis Int Veld, J. H. J. (1992). Selection of strains for probiotic use. In R. Fuller (Ed.), *Probiotics: The scientific basis* (pp. 209–224). London: Chapman e Hall.

Liew, S. L., Ariff, A. B., Raha, A. R., & Ho, Y. W. (2005). Optimization of medium composition for the production of a probiotic microorganism *Lactobacillus rhamnosus* using response surface methodology. *International Journal of Food Microbiology, 102*, 137–142.

Minolta. (1998). Precise color communication — Color control from perception to instrumentation (p. 59). Osaka: Minolta Co. Ltd.

Mousavi, Z. E., Mousavi, S. M., Razavi, S. H., Emam-Djomeh, Z., & Kiani, H. (2011). Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of Microbiology and Biotechnology, 27*, 123–128.

Nazarro, F., Fratianni, F., Sada, A., & Orlando, P. (2008). Synbiotic potential of carrot juice supplemented with *Lactobacillus* spp. and inulin or fructooligosaccharides. *Journal of Science of Food and Agriculture, 88*, 2271–2276.

O'Donnell, C. P., Tiwari, B. K., Bourke, P., & Cullen, P. J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in Food Science & Technology, 21*, 358–367.

Ouwehand, A. C., & Salminen, S. J. (1998). The health effects of cultured milk products with viable and non-viable bacteria. *International Dairy Journal, 8*, 749–758.

Pereira, A. L. F., Maciel, T. C., & Rodrigues, S. (2011). Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei*. *Food Research International, 44*, 1276–1283.

Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. *Food Research International, 43*, 1–7.

Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal, 17*, 1262–1277.

Sheehan, V. M., Ross, P., & Fitzgerald, G. F. (2007). Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. *Innovative Food Science and Emerging Technologies, 8*, 279–284.

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. (2009). Inactivation kinetics of pectin methylesterase and cloud retention in sonicated orange juice. *Innovative Food Science and Emerging Technologies, 10*, 166–171.

Tiwari, B. K., O'Donnell, C. P. O., Patras, A., & Cullen, P. J. (2008). Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *Journal of Agriculture and Food Chemistry, 56*, 10071–10077.

Valero, M., Recrosio, N., Saura, D., Muñoz, N., Martí, N., & Lizama, V. (2007). Effects of ultrasonic treatments in orange juice processing. *Journal of Food Engineering, 80*, 509–516.

Vinderola, C. G., & Reinheimer, J. A. (2000). Enumeration of *Lactobacillus casei* in the presence of *L. acidophilus*. *Bifidobacteria* and lactic starter bacteria in fermented dairy products. *International Dairy Journal, 10*, 271–275.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2006). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology, 97*, 1427–1430.