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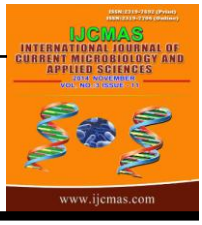

Original Research Article

A Comparative study on Probiotication of mixed Watermelon and Tomato juice by using Probiotic strains of Lactobacilli

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Keywords

Watermelon, tomato juice, probiotication *Lactobacillus fermentum*, *Lactobacillus casei*.

A B S T R A C T

This study was aimed to evaluate the suitability of watermelon and tomato juice as a raw material for production of probiotic mixed juice by growing on *Lactobacillus fermentum* and *Lactobacillus casei*. Experiments were conducted in 250 mL flasks, each containing 100 mL of mixed juice in equal proportions, sterilized for 15 min at 120ºC, inoculated separately with 24 h old broth cultures (~10^6cfu/mL), incubated at both 30°C and 37°C and analyzed for pH, acidity, sugar content and viable cell counts for a period of 72 h. They grew better at 30°C for the first 24 h with an increase in cell number (>1.8 log cfu/mL). However the viable cell counts from both the temperatures were not much different (0.7 log cfu/mL) after 72 h. Both strains produced a similar amount of titrable acidity expressed as lactic acid. But the titrable acidity produced was about two times higher at 30°C than that produced at 37°C (0.7% vs. 0.4% lactic acid). After four weeks of cold storage at 4ºC, *L. fermentum* grown at lower temperature (30°C) and *L. casei* grown at higher temperature (37°C) survived better. The addition of sucrose at the beginning of fermentation increased the amount of titrable acidity by at least two times (>1.8% lactic acid) and causing the decrease in cell viability while it was stored at 4ºC for four weeks.

Introduction

The concept of probiotic was first introduced by Elie Metchnikoff, who observed that the consumption of fermented milk could reverse putrefactive effects of the gut microflora (Metchnikoff, 1908). The application of probiotic bacteria in food for promoting health benefits has been carried out for 20 years. The increasing demand in this functional food is a response to the consumer demand for health food options (Menrad, 2002). Probiotics are living affect the host by improving its intestinal microbial balance (Fuller, 1993). Supplementation of probiotics in food products provides several health benefits, these include controlling intestinal infection, controlling serum cholesterol levels, beneficially influencing the immune system, improving lactose utilization in lactose maldigestors, and having anti-carcinogenic activity (Berner and O Donnell, 1998).

Fruit and vegetable juices have been suggested as an ideal medium for cultivating probiotic microorganisms. Fruit and vegetable juices have functional health ingredients because they inherently contain beneficial nutrients (e.g., vitamins), rich in antioxidants, dietary fibers and minerals (Yoon *et al.,* 2004). Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population. They have taste profiles that are pleasing to all age groups they are perceived as being healthy and refreshing (Luckow and Delahunty, 2004). Fruit and vegetable juices may be considered as an alternative vehicle for the delivery and incorporation of probiotics into human intestine (Tuorila and Gardello, 2002).

Because they are rich in nutrients and do not contain starter cultures that compete for nutrients with probiotics. Fruit and vegetable juices contain high amounts of sugars which could encourage probiotic growth and could easily be monitored using a Refractometer. For these reasons, fruit and vegetable juices should be examined for their ability to support probiotic and prebiotic delivery in humans.

Watermelon juice is considered as a healthy drink which is rich in lycopene, minerals and vitamins such as A, B and C. Regular consumption of water melon juice can increase blood concentration of lycopene and beta-carotene. Studies suggest that these potent antioxidants may have protective effects against heart disease and certain cancers, such as prostate, bladder, and cervical cancer (Edwards *et al.,* 2003). Tomato juice contains water (93.1%), carbohydrate (4.89%), lycopene, vitamins, minerals and is low in protein and fat (Abdel-Rahman and Abdel-Hamd, 1982). Tomato juice is well recognized as one of the healthy beverages (Suzuki *et al.,* 2002).

This study was conducted in order to determine the suitability of mixed watermelon and tomato juice as a potential raw material for growth and production of probioticated mixed juice by two proven probiotic lactobacilli (*Lactobacillus fermentum* and *Lactobacillus casei*), separately for comparison.

Materials and Methods

Preparation of substrate

Watermelon was purchased from a local vegetable market in Tirupati, India and kept at 4ºC prior to use. Juice was prepared from homogenized skinless slices by using a laboratory grinder and filtered through a muslin cloth with a sieve (0.8 to 1.1 mm pore size) to separate watermelon juice and cake containing peel and seed. Similarly fresh tomatoes were purchased from a local vegetable market in Tirupati India.

The purchased fresh tomatoes were stored in the box at room temperature for further maturation. The tomatoes were washed with tap water to remove soil and other impurities, dried at room temperature prior to use, and treated with steam for 3 to 4 min for easy peeling. The tomato fruits were processed as mentioned above to get juice.

Microorganisms

Two probiotic cultures, *Lactobacillus fermentum* (MTCC1325) and *Lactobacillus casei* (MTCC1423) were obtained from the Microbial Type Culture Collection, Chandigarh (India).

Inoculum preparation

The cultures were grown at 30 and 37°C separately for 24 h in de Man, Rogosa and Sharpe (MRS) broth and its composition is Dextrose 20.0; Meat peptone 10.0; Beef

extract 10.0; Yeast extract 5.0; Sodium acetate 5.0; Disodium phosphate 2.0; Ammonium citrate 2.0; Tween-80 1.0; Magnesium sulfate 0.1: Manganese sulfate 0.05 g/L in order to attain approximately 10^6 cfu/mL as inocula before inoculation into mixed water melon and tomato juice. Enumeration of the cells was done by plating serial dilutions of bacterial suspensions on MRS agar (Hi-media, Mumbai) plates, and incubating at 30 and 37ºC, and counting the colonies after 48 h.

Probiotication of mixed watermelon and tomato juice

Fermentation experiments were conducted in 250 mL flasks, each containing 100 mL of watermelon and tomato juice in equal proportions and sterilized for 15 min at 120ºC. All samples of mixed juice were inoculated (~10^6cfu/mL) with 24 h old cultures of the two different probiotic cultures and incubated at 30 or 37°C on a rotary shaker (120 rpm) for 72 h. Samples were taken every 24 h for chemical and microbiological analyses. In case of sucrose added mixed juice an amount of 7 and 14% (w/v) was added separately prior to sterilization.

Chemical and microbiological analyses

The pH of probioticated mixed watermelon and tomato juice was measured with a pH meter. Total acidity, expressed as percent lactic acid and was determined by titrating the mixed juice samples with 0.1 N NaOH to pH 8.2, using phenolphthalein as indicator. Sugar content was analyzed by using the method of Dubois *et al.,* (1956). Viable cell counts were determined by the standard plate method with MRS agar medium after 48 h of incubation at 37°C.

Effect of cold storage on cell viability in probiotic watermelon and tomato juice

After 72 h of fermentation, the samples of mixed watermelon and tomato juice were stored at 4°C for 4 weeks. Samples were taken at weekly intervals and the viability of probiotic cultures in the juice was determined and expressed as colony forming units (cfu/mL) by plate count method.

Statistical analysis

All fermentation experiments were conducted in triplicate and the results are expressed as mean ±S.D (standard deviation).

Results and Discussion

Viable cell counts, pH and titrable acidity of watermelon and tomato juice during 72 h fermentation period

L. fermentum and *L. casei* were found capable of growing well in sterilized mixed juice without nutrient supplementation. The time courses of lactic acid fermentation of mixed juice by these species are presented in Tables1 to 2 respectively. The results show that *L. fermentum* and *L. casei* grew significantly better at 30°C than at 37°C for the first 24 h. The increase in cell numbers was >1.6 log cfu/mL at 30°C, as compared to <0.8 log cfu/ml at 37°C. This observation is in contrary to the one reported by Ma and Marquis (1997)**,** who found that the optimum temperature for the growth of *L. casei* was 37°C, but is similar to that reported by Shamala *et al.,* (2000) , that the optimum temperature for the growth of *L. fermentum* was 30°C. Extending the fermentation beyond 24 h resulted in a significant decrease in the viable cell counts of *Lactobacilli* at 30 but not at 37°C.

However, the viable cell number of two strains at 72 h fermentation at both temperatures was not much different (<0.6 Log cfu/mL).

According to Tables 1 and 2, the mixed juice fermented at 37°C showed a significantly faster reduction in pH during the first 24 h, compared with that fermented at 30°C (1.5. vs. 0.8 units differ). However, after 48 h fermentation the pH of the juices incubated at 30 and 37°C showed nearly the same final pH, especially that of the watermelon and tomato juice fermented by *L. fermentum*. This observation agrees with Narvhus *et al.,* (1988) , who studied the production of fermented milk at 22, 30 and 37°C by lactococci and observed that products incubated at 37°C showed a faster reduction in pH early in the fermentation period, but that after 18 h the products incubated at 30 and 37°C showed the same final pH. Both strains maintained a stable pH in watermelon and to tomato juice during prolonged incubation between 24-72 h at both 30 and 37°C. This observation was different from Ostlie *et al.,* (2005), who studied the effect of temperature on metabolism of probiotic bacteria in milk and observed that after 24 h incubation *Lactobacillus reuteri* SD2112 and *Lactobacillus rhamnosus* GG caused a further decrease in pH on prolonged incubation between 24 - 48 h at 30°C, but showed a stable pH during prolonged incubation at 37°C from 24 - 48 h.

Both strains produced significantly more titrable acidity expressed as lactic acid at 30°C of fermentation than that at 37°C, though even at 37°C the cultures could utilize sugar in the juice more so than at 30°C (Tables 1 and 2). For example, *L. fermentum* produced only 0.2% acidity and reduced the pH to 4.0 after 72 h of fermentation at 37°C, but it produced 0.44% acidity and reduced the pH to 3.9 after 72 h of fermentation at 30°C. This is in contrary to what found by Ostlie *et al.,* (2005). As they reported that the amount of lactic acid produced in fermented milk by *Lactobacilli* was the highest at 37°C.

The survival of lactic acid bacteria in fermented-watermelon and tomato juices with sucrose free during cold storage

The data in Table 3 illustrates the effect of cold storage on the viability of both species of lactic acid bacteria in fermented mixed watermelon and tomato juice. *L. fermentum* grown in 30°C could survive for several weeks in the fermented mixed juice at 4°C, significantly better than that grown in 37°C. For example, the viable cell counts of *L. fermentum* grown at 30°C was still 1.8×10^6 cfu/mL after four weeks of storage at 4°C, while that grown at 37°C was reduced to 2.9×10^4 cfu/mL. In contrast, the growth temperature did not have much effect on the survival of *L. casei* during cold storage. The viable cell counts of *L. casei* was approximately 1.6×10^6 cfu/mL after 4 weeks of storage at 4°C previously incubated at either 30 or 37°C. For the maximum health benefits, not less than a million viable cells/ml of probiotic product have to be present for transfer of the probiotic effect to consumers (Lourens-Hattingh and Viljoen, 2001).

The probiotic culture should be able to multiply to reach high cell counts in the fermented product and possess a high acid tolerance to ensure high viable cell numbers during storage. The viability of probioitic organisms is dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature (Shah, 2000). Furthermore, it is also affected by lactic acid produced during production and cold storage.

Table.1 Time courses of probiotication of mixed juice of watermelon and tomato by *Lactobacillus fermentum* and *Lactobacillus casei* at 30°C

Name of the strain	Time (h)	pH	Acidity (%)	Sugar (mg/ml)	Cell number (cfu/ml)
L. fermentum	0	4.80±0.01	0.26±0.00	9.67±0.9	$3.4\pm0.4 \times 10^7$
	24	3.91±0.00	0.51±0.00	2.76±0.28	$2.7\pm0.3\times 10^9$
	48	3.90±0.00	0.47±0.01	1.83 ±0.53	$5.2\pm1.0 \times 10^8$
	72	3.20±0.00	0.44 ±0.01	1.83±0.21	$4.7\pm0.5 \times 10^8$
L. casei	0	4.75±0.04	0.26±0.00	9.37±0.77	$8.3\pm3.7 \times 10^7$
	24	3.33±0.02	0.52±0.01	4.72±0.42	$4.3\pm0.7 \times 10^9$
	48	2.89±0.00	0.51±0.04	4.77 ±1.45	$3.1\pm0.2 \times 10^8$
	72	2.51±0.00	0.53 ±0.00	4.08±0.48	$2.8\pm0.2 \times 10^8$

Mean and standard deviation for n=3

Table.2 Time courses of probiotication of mixed juice by *L. fermentum L. casei* at 37°C

Name of the strain	Time (h)	pH	Acidity (%)	Sugar (mg/ml)	Cell number (cfu/ml)
L. fermentum	0	5.33±0.03	0.18±0.01	13.10±4.5	$4.6\pm1.5 \times 10^7$
	24	4.01±0.05	0.25±0.02	3.54±0.83	$2.1\pm0.5 \times 10^8$
	48	3.96±0.12	0.21±0.00	3.50 ±0.90	$1.7\pm0.2 \times 10^8$
	72	3.67±0.13	0.22 ±0.01	3.23±0.52	$2.3\pm0.5 \times 10^8$
L. casei	0	4.98±0.04	0.18±0.01	20.70±4.99	$2.7\pm0.1 \times 10^7$
	24	3.75±0.01	0.32±0.02	1.68±0.13	$4.3\pm0.8 \times 10^7$
	48	3.91±0.03	0.29±0.01	0.99 ±0.14	$2.5\pm0.1 \times 10^8$
	72	3.60±0.01	0.32 ±0.00	0.45±0.07	$9.4\pm2.2 \times 10^8$

Mean and standard deviation for n=3

Table.3 Effect of cold storage at 4°C on the viability of lactic cultures in fermented mixed juice, prepared at different temperatures without sucrose

Time	Cell viability at 30 °C(cfu/ml)		Cell viability at 37 °C(cfu/ml)	
weeks	***L. fermentum***	***L. casei***	***L. fermentum***	***L. casei***
0	$4.7\pm0.5 \times 10^8$	$2.8\pm0.2\text{x}10^8$	$2.3\pm0.5\ \text{x}10^8$	$9.4\pm2.2 \times 10^8$
1	$3.0\pm1.0 \times 10^7$	$4.7\pm0.5\text{x}10^6$	$1.4\pm0.2\text{x}10^7$	$1.5\pm0.4 \times 10^7$
2	$2.2\pm0.1\times1\ 0^6$	$2.47\pm0.04\text{x}10^6$	$9.2\ \pm0.9\ \text{x}10^5$	$1.3\pm0.08\ \times10^7$
3	$1.9\pm0.2 \times 10^6$	$2.0\pm0.1\ \text{x}10^6$	$7.1\pm0.6\ \text{x}10^4$	$6.5\pm0.3 \times 10^6$
4	$1.9\pm0.1 \times 10^6$	$1.9\pm0.06\text{x}10^6$	$3.0\pm0.3\ \text{x}10^4$	$1.7\pm0.03 \times 0^6$

Mean and standard deviation for n=3

Table.4 Time courses of probiotication of mixed juice by *Lactobacillus fermentum, Lactobacillus casei,* at 30°C, with addition of 7% sucrose

Name of the strain	Time (h)	pH	Acidity (%)	Sugar (mg/ml)	Cell number (cfu/ml)
L. fermentum	0	4.81±0.00	0.26±0.03	$1.2±0.9 \times 10^8$	4.81±0.00
	24	3.53±0.02	0.76±0.04	$5.3±0.8 \times 10^9$	3.53±0.02
	48	3.29±0.00	1.18±0.00	$2.8±0.2 \times 10^8$	3.29±0.00
	72	3.27±0.01	1.20 ±0.05	$3.7±0.6 \times 10^8$	3.27±0.01
L. casei	0	4.81±0.00	0.26±0.00	$9.0±0.9 \times 10^7$	4.81±0.00
	24	3.81±0.03	0.55±0.04	$8.0±0.5 \times 10^9$	3.81±0.03
	48	3.40±0.01	0.91±0.01	$4.0±0.6 \times 10^9$	3.40±0.01
	72	3.31±0.01	1.14 ±0.05	$4.8±0.4 \times 10^9$	3.31±0.01

Mean and standard deviation for n=3

Table.5 Time courses of probiotication of mixed juice by *Lactobacillus fermentum, Lactobacillus casei* at 30°C, with addition of 14% sucrose

Name of the strain	Time (h)	pH	Acidity (%)	Sugar (mg/ml)	Cell number (cfu/ml)
L. fermentum	0	4.80±0.02	0.26±0.03	$2.7±0.7 \times 10^8$	4.80±0.02
	24	3.50±0.02	0.72±0.04	$6.1±0.6 \times 10^9$	3.50±0.02
	48	3.25±0.01	1.18±0.01	$7.5±0.5 \times 10^8$	3.25±0.01
	72	3.20±0.01	1.41±0.00	$6.1±0.8 \times 10^8$	3.20±0.01
L. casei	0	4.76±0.02	0.25±0.02	$8.3±1.6 \times 10^7$	4.76±0.02
	24	3.78±0.03	0.55±0.04	$6.5±0.8 \times 10^9$	3.78±0.03
	48	3.38±0.01	0.92±0.01	$5.1±0.4 \times 10^9$	3.38±0.01
	72	3.27±0.01	1.25 ±0.05	$6.5±0.5 \times 10^9$	3.27±0.01

Mean and standard deviation for n=3

Table.6 Effect of cold storage on the viability of lactic cultures in fermented mixed juice, prepared at 30 and 37 °C with sucrose

Time	Cell viability at 30 °C(cfu/ml)		Cell viability at 37 °C(cfu/ml)	
weeks	*L. fermentum*	*L. casei*	*L. fermentum*	*L. casei*
0	$4.7±0.6 \times 10^8$	$4.8±0.4 \times 10^9$	$6.2±0.8 \times 10^8$	$6.5±0.5 \times 10^9$
1	$3.1±0.9 \times 10^7$	$3.1±0.2 \times 10^6$	$3.7±0.6 \times 10^6$	$3.7±0.1 \times 10^6$
2	$2.3±0.5 \times 10^6$	$2.9±3.4 \times 10^6$	$3.6±0.7 \times 10^6$	$2.3±0.6 \times 10$
3	$1.8±0.3 \times 10^6$	$2.2±3.4 \times 10^6$	$2.0±0.3 \times 10^6$	$2.0±1.3 \times 10^6$
4	$1.5±0.4 \times 10^6$	$1.8±0.3 \times 10^6$	$1.4±0.6 \times 10^6$	$1.1±0.9 \times 10^6$

Mean and standard deviation for n=3

Growth and acid production of lactic acid bacteria in added- sucrose watermelon and tomato juice during 72 h fermentation period

When sucrose was added to the mixed juice and fermentation conducted at 30°C, both strains produced significantly more titrable acidity expressed as lactic acid than the sucrose free juice (Table 1, 4 and 5).

The increase of acidity at 72 h fermentation was approximately 3 times higher in sucrose added watermelon and tomato juice fermented with *L. fermentum* (Table 1, 4 and 5) and nearly 2.6 times higher in sucrose added juices fermented with *L. casei* (Table 2, 4 and 5), compared with the sucrose free juice. Greater reduction of pH for the first 24 h of fermentation in the sucrose added watermelon and tomato juice was also observed.

After 48 h, the pH of all fermented sucrose added watermelon juice was reduced below 3.6. Haddadin (2005) also reported the significant effect of sugar on the acidification of milk. He found that the addition of glucose and galactose caused the faster acidification of milk fermented by *L. fermentum* and *L. casei*, compared with the control. On the other hand, the addition of sucrose did not support good growth of *L. fermentum*, same as the results reported by Shamala *et al.,* (2000).

However, sucrose supported the growth of *Lactobacillus casei* in this study. Its cell number at 72 h of fermentation was 4.6 – 6.3 × 10^9 cfu/mL in sucrose added watermelon and tomato juice, compared with 2.6 × 10^8 cfu/mL in sucrose free juice.

The survival of lactic acid bacteria in fermented sucrose added watermelon and tomato juice during cold storage

Both strains were not able to survive well at the lower pH and higher acidity conditions in fermented sucrose added watermelon and tomato juice at 4°C. As can be seen from Table 6, the viable cell counts of both strains reduced to approximately 10^4 cfu/mL in fermented sucrose - added juice after 4 weeks of cold storage at 4°C, compared with 10^6 cfu/mL in sucrose-free juice (Table 3).

Sensory evaluation

Further the fermented mixed juice taste, flavor and acceptability was tested and compared with that of probiotic branded yogurt (Nestle). Fermented juices with sugar had more acceptable taste and flavor than the sugar free juice. According to Luckow and Delahunty (2004), the sensory characteristic of probiotic blackcurrant juice was perfumey and dairy in odor and sour and savory in flavor. These off flavors also occurred in watermelon and tomato juice. However, when sucrose was added at the beginning of fermentation, these flavors seemed to be reduced and the taste was more acceptable (personal observation).

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