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Processing of Probiotic Pomegranate Juice Using Lactic Acid Bacteria

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Abstract: ***The study was undertaken for production of probiotic pomegranate juice through its fermentation by two strains of lactic acid bacteria: Lactobacillus plantarum & L. acidophilus. Pasteurization of freshly prepared pomegranate juice at 80°Cfor 15 min was conducted to decrease the microbial population to below the detection limit. Fermentation was carried out at 37°C for 72 hrs. The parameters like, microbial population, pH, titrable acidity, and sugar (glucose) were measured during the fermentation period and viability of all the strains was also determined during the storage time at 4°C within week.***
The results indicated that L. plantarum & L. acidophilus decreased the pH sharply at initial stages of fermentation, sugar consumption was also higher and better microbial growth was also observed for these two strains during fermentation. L. acidophilus showed higher viability during the storage time than L. plantarum. Viable cells remained at their maximum level within 2 weeks but decreased dramatically after 3 weeks. The Pomegranate juice proved to be a suitable media for production of a probiotic drink.
Keywords: - Pasteurization, Pomegranate juice, Probiotic, Fermentation, Lactobacillus plantarum, Lactobacillus acidophilus,

I. INTRODUCTION

Probiotic has been defined as alive or dead organism from *Lactobacillus, Streptococcus, Enterococcus, Bacillus* and *Saccharomyces* genera as well as microbial fermentation products, nucleotides and its metabolizable products, metabolites and organic acids (Ouwehand *et al.*, 1999). Probiotics are live microorganisms that are similar to beneficial microorganism found in the human gut. They are also called “Friendly bacteria or good bacteria”. Certain intestinal microbes are known to produce vitamins and they are non-pathogenic, their metabolism is non-putrefactive, and their presence is correlated with a healthy intestinal flora. The metabolic end products of their growth are organic acids (lactic and acetic acids) that tend to lower the pH of the intestinal contents, creating conditions less desirable for harmful bacteria. Probiotics also influence other protective functions of the intestinal mucosa including synthesis and secretion of antimicrobial peptides. The Gastro Intestinal tract also serves as large mucosal surfaces that bridge the gap between ‘inside the body’ and ‘outside the body’. Along this mucosal interface, microbes and foreign antigens colonizing or passing through the Gastro Intestinal tract interact with important components of the immune system. Normal microbial inhabitants of the Gastro Intestinal tract also reinforce the barrier function of the intestinal lining, decreasing ‘translocation’ bacteria from intestine into the blood stream. This function has been suggested to decrease infections. (Pascual *et al.*, 1996)

Fruits and vegetables as functional foods are enriched with minerals, vitamins, dietary fibres, and antioxidants (Phytochemicals). It has been suggested that fruit juices could serve as suitable media for cultivating probiotic bacteria (Mattila *et al.*, 2002).

Pomegranate (*Punica granatum, punicaceae*) is known to have health-promoting properties with antimicrobial, antiviral, anticancer, antioxidant and antimutagenic effects (Negi *et al.*, 2003). The fresh juice contains 85.4% water and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid, proteins and has been reported to be a rich source of antioxidants. These antioxidants are more potent, on a molar basis, than many other antioxidants including vitamin C, vitamin E, coenzyme Q-10 and alpha-lipoic acid (Aviram *et al.*,2002).

The Microorganisms from several selected bacterial genera such as *Bacillus coagulants, Bifidobacterium, Enterococcus faecium, Lactobacillus casei, Lactobacillus acidophilus, Streptococcus , Lactococcus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii* are currently utilized as probiotics (Yoon *et al.*, 2004).

Lactic acid bacteria (LAB) have long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic and shelf-life characteristics. *Lactobacillus acidophilus* is a member of one of the eight main genera of Lactic acid bacteria.

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L. acidophilus is characterized as being a rod shaped motile bacteria grows in the presence of oxygen. *L. acidophilus* is also characterized as a homo fermentative that only produces lactic acid as its sole products. *L. acidophilus* is important in the fermentation of many foods from dairy products to fruits and vegetables. Fermentation occurs when bacteria break down sugars and carbohydrates to produce alcohol, carbon dioxide and lactic acid *Lactobacilus* are able to live in highly acidic environments of pH 4-5 , *L. acidophilus* can help maintain a healthy balance of intestinal flora by increasing acidity of the intestine, killing off harmful bacteria. *L. acidophilus* was demonstrated to have antimicrobial effects against pathogen.

L. plantarum is rod shaped. Its genome is the largest among all lactic acid bacteria. *L. plantarum* is also commonly used for food fermentation and turn sugars into lactic acid or alcohol (heterofermentative). It is a facultative anaerobic bacterium. It can convert oxygen into hydrogen peroxide, which gives itself a high hydrogen peroxide tolerance. *L. plantarum* can survive low pH of the stomach and it can enhance the intestinal integrity, metabolic activity of intestinal cells and stimulate immune responses.

As the market for functional food grows and probiotic microorganism conferring health benefit on the host so these products are gaining a great attention by the consumers. It is playing significant role improvising consumer health.

II. MATERIALS AND METHODS



Isolation & Collection of Lactic acid bacteria

Preparation and heat treatment and fermentation of pomegranate juice



Determination of pH, lactic acid content, reducing sugar and TBC in probiotic pomegranate juice



Effect of fermentation time on juice



probiotic pomegranate juice by bacteria under pasteurized condition

Survival of bacteria in fermented pomegranate juice during cold storage

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A. Bacterial Strain And Culture Used

*1) Lactobacillus acidophilus MCCB*0110

2) Lactobacillus plantarum MCCB0157

B. Collection and Maintenance of Microbial Strains and Culture

Lactic acid bacterial isolates namely *Lactobacillus acidophilus* and *Lactobacillus plantarum* were collected from MCCB (Microbiological Culture Collection Bank) and these cultures were revived in MRS broth and they were then sub cultured on MRS agar slants and incubated at 30°C for 24-48 hrs. The cultures were maintained at 4°C and for each trial 24 hrs. Old broth cultures were used.

C. Preparation of Pomegranate Juice

The purchased fresh pomegranate was washed with tap water to remove soil and other impurities, dried at room temperature prior to use. Pomegranate fruits were peeled and the skin covering, removed manually. The pomegranate fruits were ground and filtered to separate pomegranate juice.

D. Heat Treatment And Fermentation Of Pomegranate Juice

The pomegranate juice was pasteurized for 20 min at 80°C. 24 hours cultivation if lactic acid bacteria in the MRS broth were carried out at 30°C. For obtaining initial cell density of 10^7 cfu/ml in the final juice, 15 ml of cultivated MRS broth was centrifuged at 4,000g for 10 min and the biomass was introduced into the juice (150 ml). The juice was then incubated at 30°C for 72 hours and samples were taken every 24 hours for determination of pH, acidity, sugar and total bacterial count.

E. Determination Of Ph, Lactic Acid Content, Reducing Sugar (Glucose), And Total Bacterial Count In Probiotic Pomegranate Juice

Sample was taken at every 24 hours intervals for determine the probiotic properties pomegranate juice.

F. pH Measurement

pH was measured with a pH meter at every 24 hours, the calibration of pH meter was assured so that correct result could be measured.

G. Total Acidity

Total acidity was determined by the fruit juice sample (5ml) was homogenized in distilled water (20ml) and filtered through what man No.1 filtered paper. Two-three drops of phenolphthalein were added 20 ml of the filtrate as indicator and titrated against 0.05M NaOH to the end point of phenolphthalein. Titrable acidity was expressed as g lactic acid /100g of juice and calculated using the following formula.

$$\text{Titrable acidity (g/100g)} = \frac{\text{volume of NaOH(ml) used in titration X MNaOH x } 0.09\times 100}{\text{ml juice sample}}$$

Where, TA = titrable acidity; MNaOH = Molarity of NaOH used; ml NaOH = amount (in ml) of NaOH used; 0.09 = equivalent weight of lactic acid

H. Sugar Content

Sugar content was analyzed as glucose by phenol sulphuric acid method ,phenol sulphuric acid method was conducted into boiling tube. Sample was hydrolysed with 2.5N HCl and neutralized it with the solid sodium carbonate until the effectiveness ceases. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. '0' serves as blanks and volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water. Sample was centrifuged and collected supernatant and took aliquots for analysis. Sample was pipette out and then added phenol solution, 96% sulphuric acid to each tube. Sample was placed in a water bath at 25-30°C for 20 min, read the colour at 490 nm. Standard graph was drawn by concentration of the standard on the x axis versus absorbance on the y axis.

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I. Viable cell counts (cfu/ml)

Viable cell counts (cfu/ml) were determined by the standard plate method with *Lactobacilli* MRS (Man-Rogosa Sharp Agar) medium. Serial dilutions were made in ringer's solution and diluted juice sample was mixed with the medium (MRS). The dishes were incubated at 30°C for 24 to 48 hours and the bacterial colonies were recorded.

J. Effect Of Cold Storage On Cell Viability In Probiotic Pomegranate Juice

After 72 hours of fermentation at 30°C, the fermented samples (25 ml) were stored at 4°C for 4 weeks. Samples were taken at weekly intervals, and viability of probiotic cultures in probiotic pomegranate juice was determined. Viable cell counts (cfu/ml) were determined by the standard plate method with *Lactobacilli* MRS (Man-Rogosa Sharp Agar) medium. Serial dilutions were made in ringer's solution and diluted juice sample was mixed with the medium (MRS). The dishes were incubated at 30°C for 24 to 48 hours and the bacterial colonies were recorded.

K. Statistical Analysis

The data recorded during the course of investigation were subjected to two way anova and conclusions were drawn accordingly. (Fisher and Yates, 1990)

Table 1: Skeleton of ANOVA table

Source of variation	df	SS	MSS	F Cal	F Tab at 5%
Due to replications	(r-1)	RSS	RSS (r-1)	MSS(r) EMS	
Due to treatments	(t-1)	TrSS	TrSS (t-1)	MSS(t) EMS	
Due to error	(r-1)(t-1)	ESS	ESS (r-1)(t-1)		
Total	(rt-1)	TSS			

Where,

Standard Error Deviation (SEd)

Standard error of mean was calculated by the following formula:

SEd = $\sqrt{\frac{2 \times M}{n}}$

r

Critical differences (CD)

Critical difference was calculated by the following formula:

CD = SEd x 't' error degree of freedom at 5%

r = Number of replication

df = Degree of freedom

SS = Sum of squares

RSS = Sum of squares due to replication

TrSS = Sum of squares due to treatment

TSS = Total sum of squares

ESS = Error sum of squares

MSS(r) = Mean sum of squares due to replication

MSS (t) = Mean Sum of squares due to treatment

EMS = Error mean sum of squares

SEd = Standard error deviation

III. RESULT AND DISCUSSION

A. *Production of Probiotic Pomegranate juice by L. acidophilus under Pasteurized Condition*

Lactic acid bacteria, (*L.acidophilus, L.plantarum*) used in this study was capable of growing well on sterilized juices without any nutrient supplementation. Pasteurization was carried out at 80°C for 15 min and 20 min, no significant changes were detected in sugar, acidity, and pH. Heat treatment (Pasteurization) is generally used to eliminate or reduce the contaminant microorganisms. The main requirement of heat treatment is to ensure the microbial safety of the product. The time course of lactic acid fermentation of pomegranate juices by *Lactobacillus* spp. is presented in Table 4.1 and 4.2.

Table 2: Effect of fermentation time on pomegranate Juice by *L. acidophilus* (Pasteurization at 80° C for 15 min):

Time (h)	pH	Acidity (g/100g)	Sugar (mg/ml)	CFU/ml×10^7
0	3.09	2.88	0.494	9.9
24	3	3.16	0.394	17 .5
48	2.90	3.52	0.208	14. 5
72	2.50	3.60	0.19	12.5

Due to pH: $F_{(cal)}$, = 243.69> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.050
Due to Acidity: $F_{(cal)}$, = 5.894> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 4.12
Due to Sugar: $F_{(cal)}$, = 711.13> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.017
Due to CFU: $F_{(cal)}$, = 540.72> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.616

Table 3: Effect of fermentation time of pomegranate Juice by *Lactobacillus acidophilus* (Pasteurization at 80°C for 20 min)

Time (h)	pH	Acidity (g/100g)	Sugar (mg/ml)	CFU/ml ×10^7
0	3.41	2.16	0.566	12.5
24	3.35	2.44	0.438	10.0
48	3.10	2.59	0.266	9.8
72	3.00	2.73	0.246	9.5

Due to pH: $F_{(cal)}$, = 112.87> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.55
Due to Acidity: $F_{(cal)}$, = 19.483> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.166
Due to Sugar: $F_{(cal)}$, = 344.54> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.024
Due to CFU: $F_{(cal)}$, = 466.85> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.683

B. *Variation in pH of Pomegranate Juice During Fermentation*

Although the Pomegranate juice had an initial pH value of 3.09 in 15 min pasteurized juice and 3.41 in 20 min pasteurized juice sample. Table 4.1 and 4.2 show the lactic acid cultures actively fermented the pomegranate juice and lowered the pH as low as 2.50 and 3.0 after 72 hours fermentation. According to Figure 4.1, the pomegranate juice pasteurized for 15 min shows faster reduction in pH after 48 hours, compared with juice pasteurized for 20 min. Babu *et al.* (1992) reported that addition of tomato juice to skimmed milk stimulated the growth of *L. acidophilus* and resulted in higher viable counts, shorter generation time, and improved sugar utilization with more acid production and lower pH.

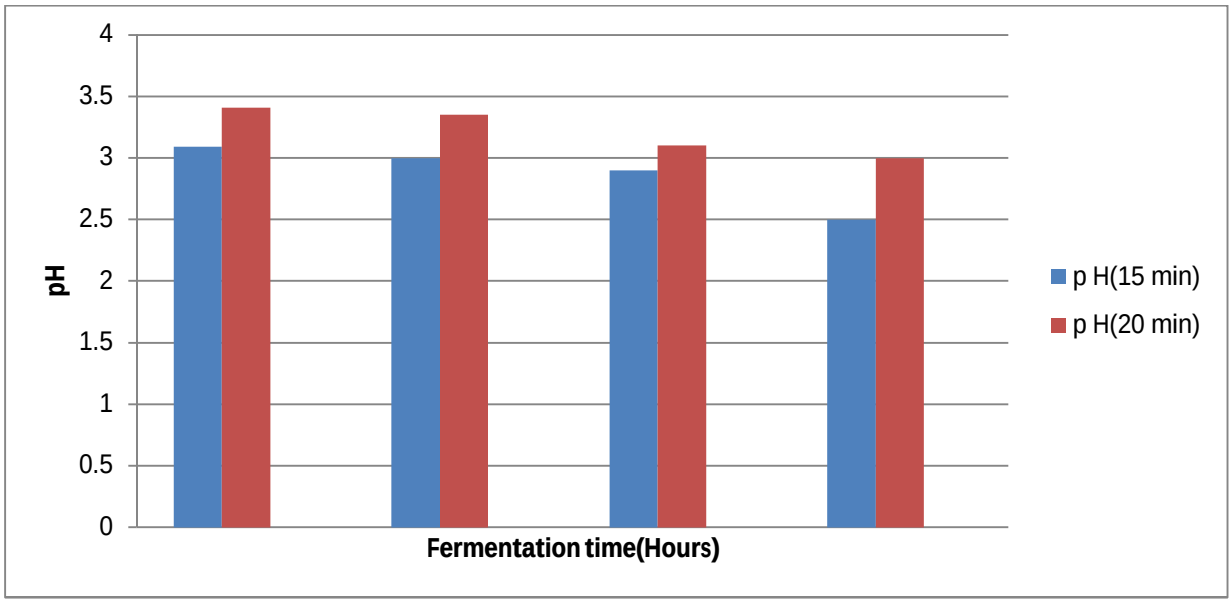


Figure 1: Variation in pH of pasteurized pomegranate juice during fermentation by *L. acidophilus*

C. Variation in Acidity Expressed as lactic acid of Pomegranate juice During Fermentation by L. Acidophilus

During fermentation of pomegranate juice, production of lactic acid was determined. Result indicated that the lactic acid produced by all the strains and its concentration increased as the fermentation commenced. Figure 4.2 shows the changes in acidity during pomegranate juice fermented by *L. acidophilus*. Although the Pomegranate juice had an initial acidity 2.88 in 15 min pasteurized juice and 2.16 in 20 min pasteurized juice and its concentration was increased after 48 hours fermentation. *L.acidophilus* produced significantly more titrable acidity in 15 min of pasteurized juice than 20 min of pasteurized juice. According to Holzapfel and Schillinger (2000), acid tolerance is an important probiotic trait for surviving during fermentation in food medium. De vries and Stouthamer (1967) reported that strains metabolized citric acid as the major carbon source available in pomegranate juice. Since lactic acid is recognized as the main metabolite produced by lactic acid bacteria.

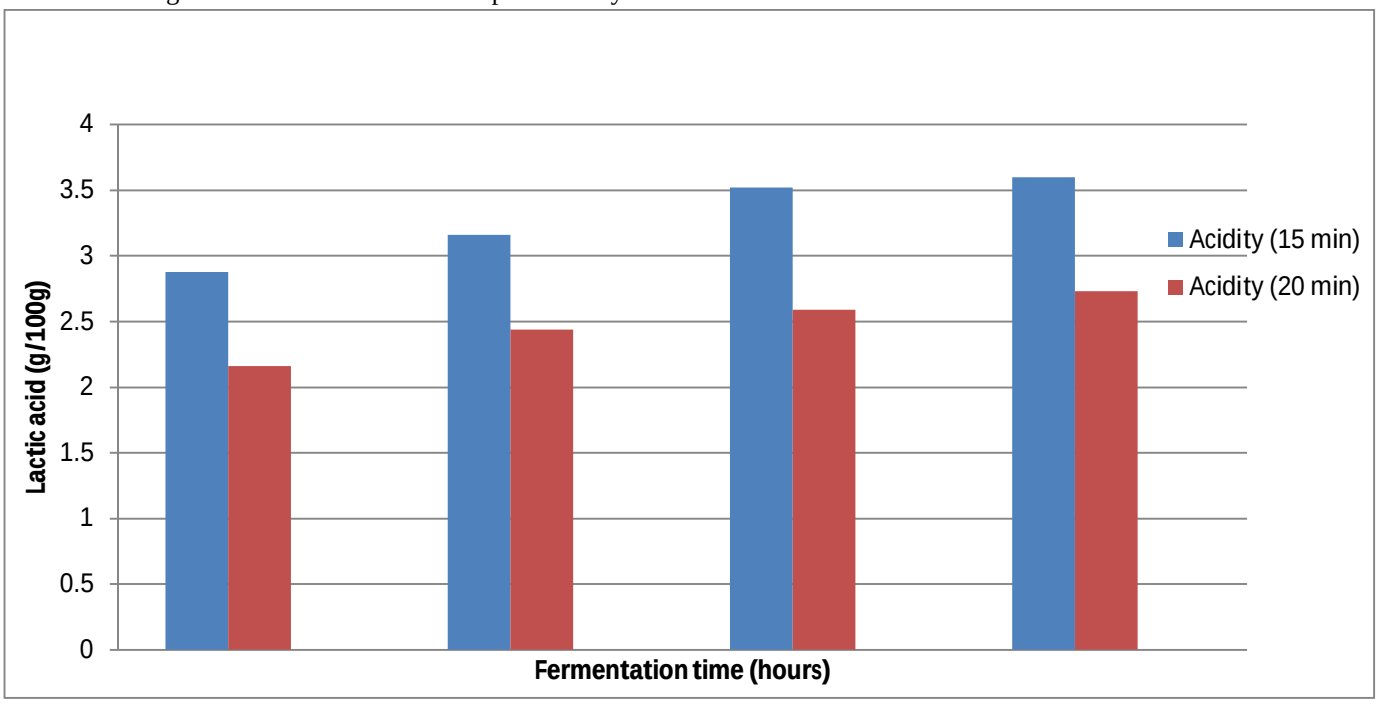


Figure 2: Change in Titrable acidity of pasteurized pomegranate juice during fermentation by *L. acidophilus*

D. Variation in glucose concentration of pomegranate juice during fermentation L. acidophilus

Determination of glucose concentration in pomegranate juice was done during fermentation. Changes in sugar content during pomegranate juice fermented by *L. acidophilus* are given in Figure 4. 3. *L. acidophilus* rapidly fermented pomegranate juice and reduced the level of sugar. The Glucose concentration was decreased from 0.494 mg/ml to 0.19mg/ml in 15 min pasteurized juice and 0.566 mg/ml to 0.246 mg/ml in 20 min pasteurized juice after 48 hrs fermentation. *L. acidophilus* utilized significantly more glucose in juice for 20 min of pasteurization than 15 min. In the research of Tezcan *et al.* (2009) glucose and fructose were dominant sugars in pomegranate juice. The concentration of glucose reduced significantly in comparison to fructose between hours 48 an 72 of the fermentation.

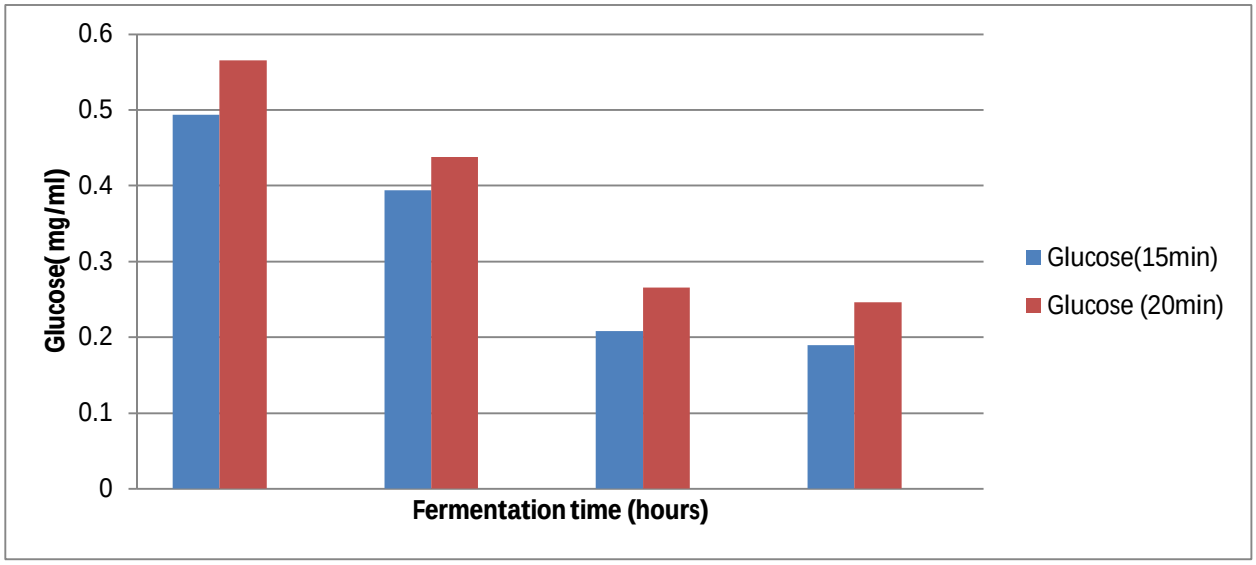


Figure 3: Variation in Glucose concentration of pasteurized pomegranate juice during fermentation *L. acidophilus*

E. Variation in microbial population of pomegranate juice during fermentation L. acidophilus

Changes in viable cell count during pomegranate juice fermented by *L. acidophilus* w grew rapidly in pasteurized pomegranate juice and reached a viable cell population 12.5 x10^7 in 15 min pasteurized juice and 9.5 x10^7in 20 min pasteurized juice after 48 hours fermentation at 30°C. Shamala *et al.* reported that the optimum temperature for growth of *L. acidophilus* was 30°C. Extending the fermentation beyond 48 hours resulted in a significant decrease in the viable cell counts of lactic acid bacteria at 30°C. Yanez *et al.* (2008) also reported that low pH of the medium can lead to the decrease in maximum growth rate and extended length of the lag phase. This stage was clearly detectable for all the strains during which metabolic activity including sugar consumption and acid production is minor and the bacterial cell try to adapt with the new conditions.

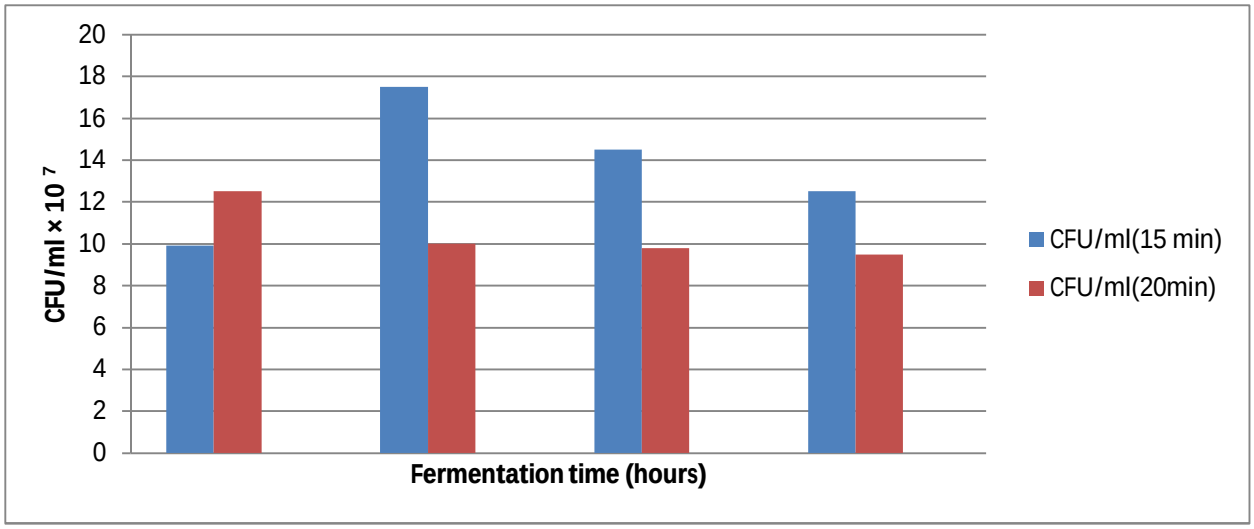


Figure 4: Growth of *L. acidophilus* during fermentation at 30°C in pasteurized pomegranate juice.

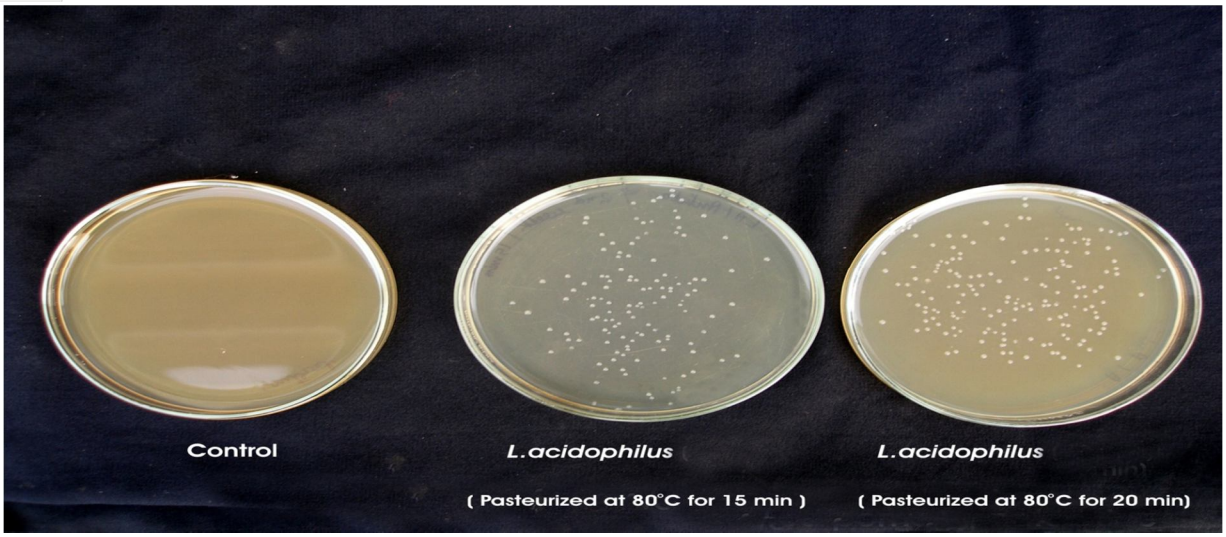


Fig No. 4.1: Growth of *L. acidophilus* in pasteurized pomegranate juice during fermentation at 30°C for 0 hour

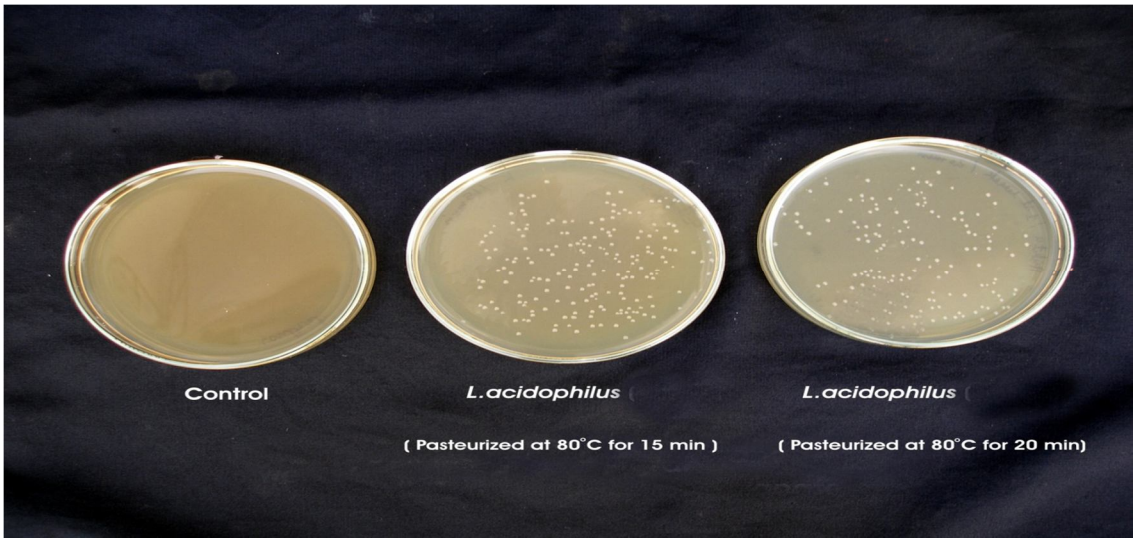


Fig No. 4.2 Growth of *L. acidophilus* in pasteurized pomegranate juice during fermentation at 30°C for 24 hours

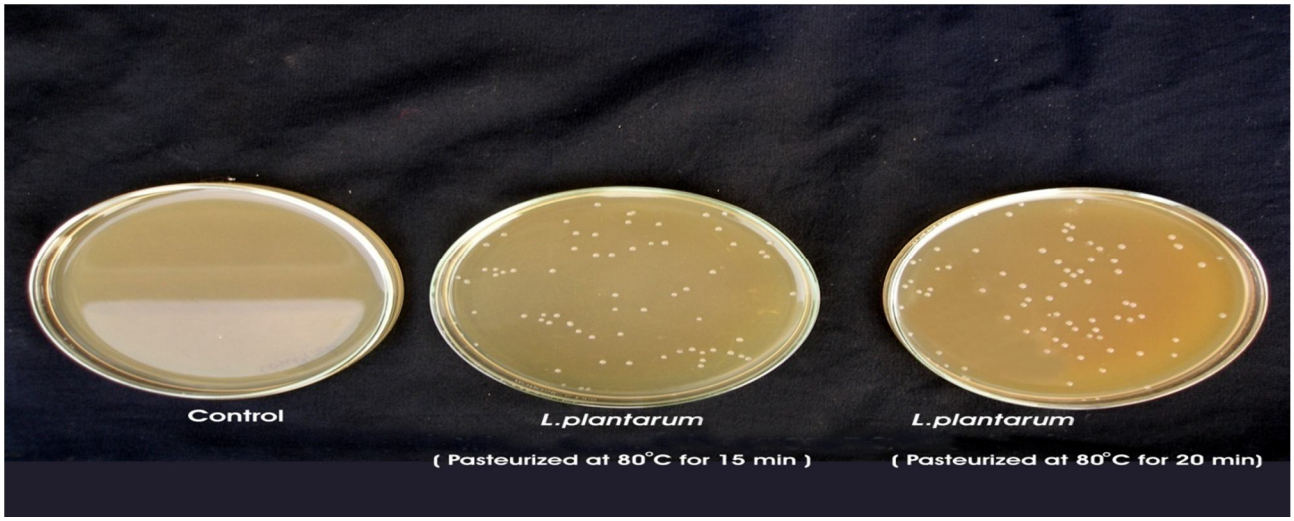


Fig No. 4.3 Growth of *L. acidophilus* in pasteurized pomegranate juice during fermentation at 30°C for 48 hours

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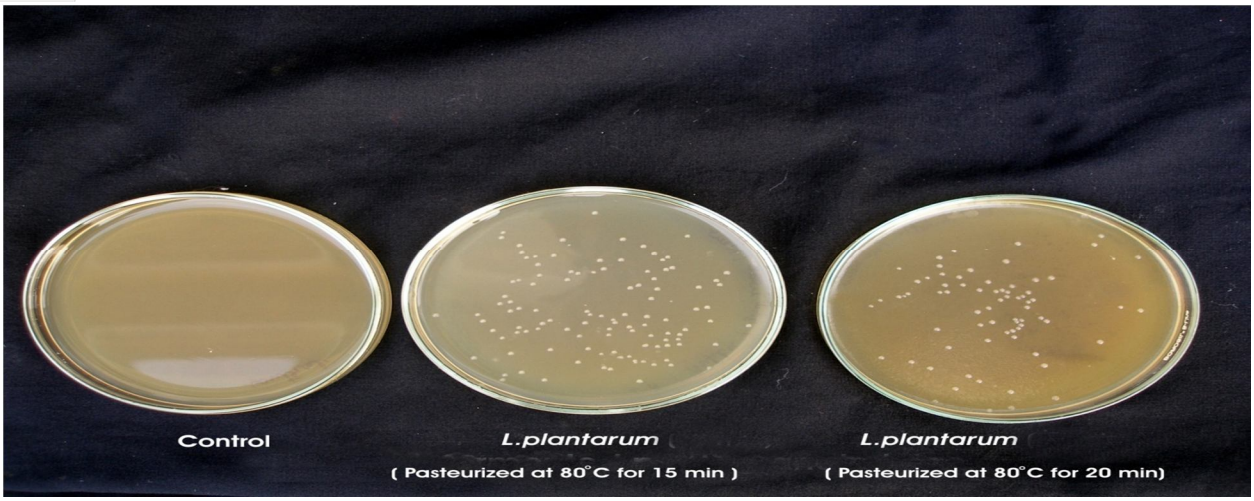


Fig No. 4.4 Growth of *L. acidophilus* in pasteurized pomegranate juice during fermentation at 30°C for 72 hours

F. Production of Probiotic pomegranate juice by L. plantarum under pasteurized condition

Effect of fermentation time of pomegranate Juice by *Lactobacillus plantarum (*Pasteurization at 80°C for 15 min and 20 min) is presented in the Table 4.3 and 4.4. Pasteurization was an appropriate method to reduce the contaminant microorganisms to acceptable level while remaining relevant from the food safety stand point.

Table 5: Effect of fermentation time of pomegranate Juice by *L. plantarum* (Pasteurization at 80°C for 15 min)

Time (h)	pH	Acidity (g/100g)	Sugar (mg/ml)	CFU/ml×10^7
0	3.09	1.29	0.122	6.8
24	3.3	1.87	0.10	5.5
48	3.2	1.87	0.084	7.5
72	3.0	2.59	0.082	4.2

Due to pH: $F_{(cal)}$, = 54.166> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.85
Due to Acidity: $F_{(cal)}$, = 63.742> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.200
Due to Sugar: $F_{(cal)}$, = 0.571> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.073
Due to CFU: $F_{(cal)}$, = 34.90> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.740

Table 5A: Effect of fermentation time of pomegranate Juice by *L. plantarum* (Pasteurization at 80° C for 20 min)

Time (h)	pH	Acidity (g/100g)	Sugar (mg/ml)	CFU/ml×10^7
0	3.41	1.29	0.254	4.8
24	3.2	1.87	0.166	5.3
48	3.1	2.59	0.054	5.5
72	3.0	2.73	0.038	3.9

Due to pH: $F_{(cal)}$, = 51.47> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.071
Due to Acidity: $F_{(cal)}$, = 36.664> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.331
Due to Sugar: $F_{(cal)}$, = 11.44> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.90
Due to CFU: $F_{(cal)}$, = 7.15> $F_{(tab)}$ =4.76(5% and 3 degree of freedom)–Significant; CD= 0.800

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G. Variation in pH of pomegranate juice during fermentation by L. plantarum

Although the Pomegranate juice had an initial pH value of 3.09 in 15 min pasteurized juice sample and 20 min pasteurized juice sample. The lactic acid cultures actively fermented the pomegranate juice and lowered the pH as low as 3 after 72 hours fermentation.

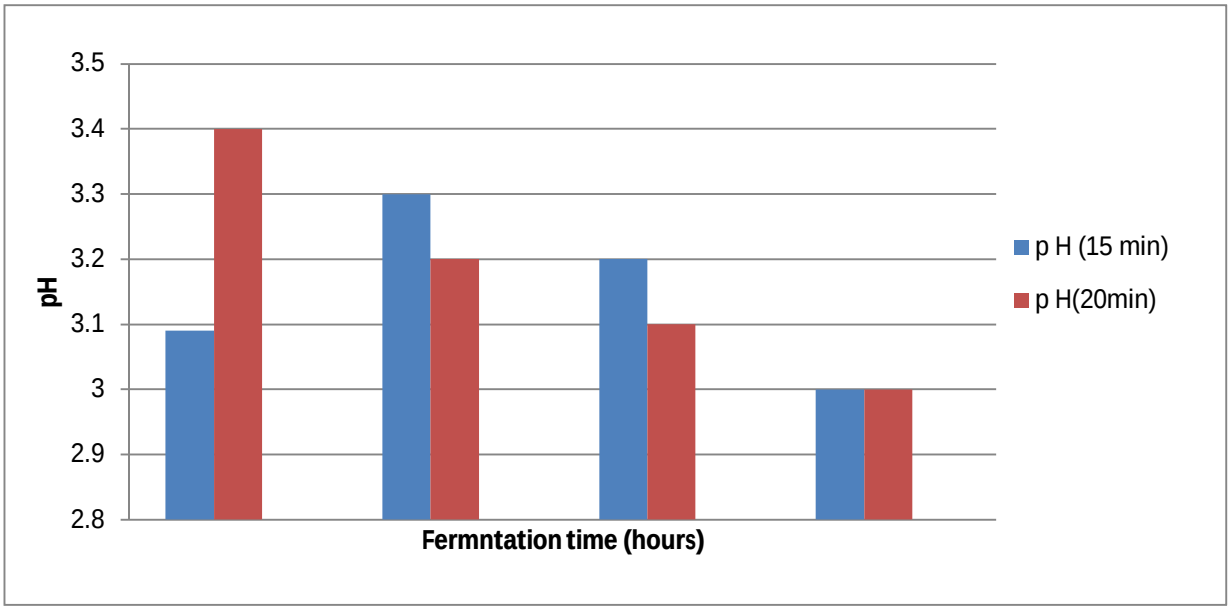


Figure 7: Variation in pH of pasteurized pomegranate juice during fermentation by *L. plantarum*

H. Variation in acidity expressed as lactic acid of pomegranate juice during fermentation by L. plantarum

Pomegranate juice had same initial acidity 1.29 in 15 min pasteurized juice and its concentration was increased after 48 hours fermentation. Figure 4.6 shows the changes in acidity during pomegranate juice fermented by *L. plantarum*. *L. plantarum* produced significantly more titrable acidity in 15 min pasteurized juice than 20 min pasteurized juice. Sindhu and khetrapaul (2001) was reported that probiotic fermentations of indigenous food mixture containing tomato pulp using *L.plantarum* showed a decreased of pH, increased of acidity, and improvement of digestibilities of starch and protein.

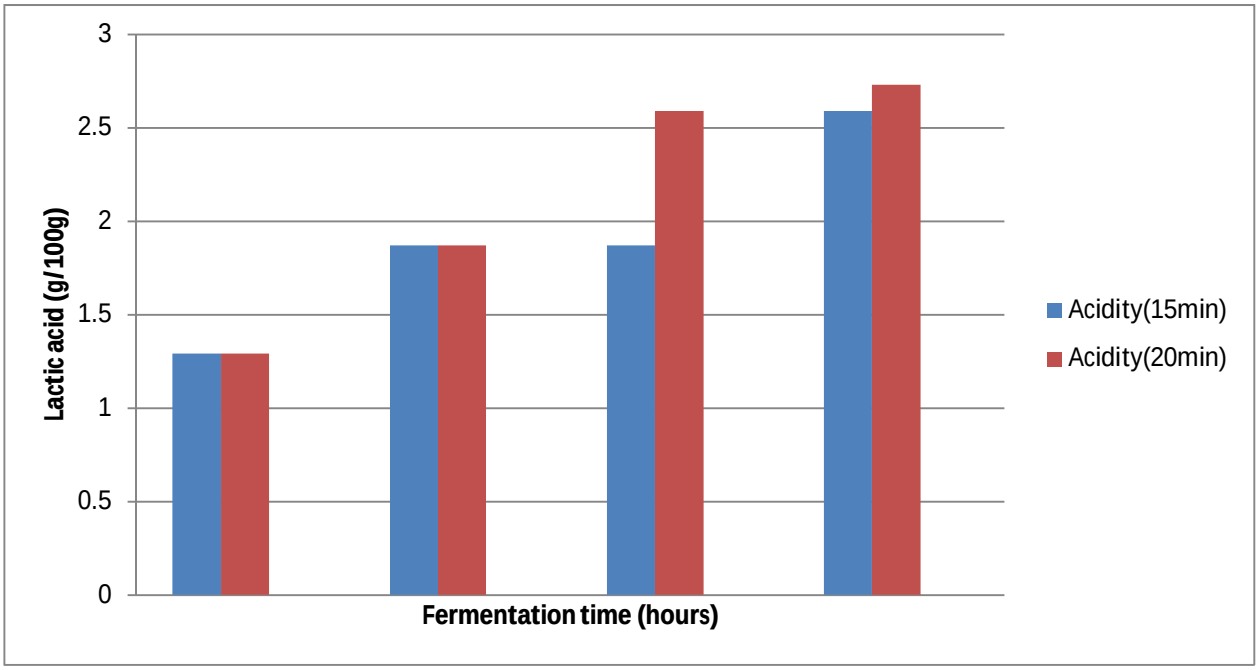


Figure 8: change in Titrable acidity of pasteurized pomegranate juice during fermentation *by L. Plantarum*

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I. Variation in Glucose Consumption of Pomegranate Juice During Fermentation

Determination of glucose concentration in pomegranate juice was done during fermentation. Changes in sugar content during pomegranate juice fermented by *L. plantarum* are given in Figure 4.7. *L. plantarum* rapidly fermented pomegranate juice and reduced the level of sugar. Glucose concentration was decreased from 0.122 mg/ml to 0.082 mg/ml in 15 min pasteurized juice sample and 0.254mg/ml to 0.038 mg/ml in 20 min pasteurized juice after 48 hrs fermentation. *L. plantarum* utilized significantly more glucose in juice for 20 min of pasteurization than 15 min. Hou *et al.* (2000) proved that metabolism of carbohydrates by *Lactobacillus* varies from strain to strain and depends on the substrate and even on the fermentation time and Wang *et al.* (2003) also reported that glucose is a very good carbon and energy source for *Lactobacilli.*

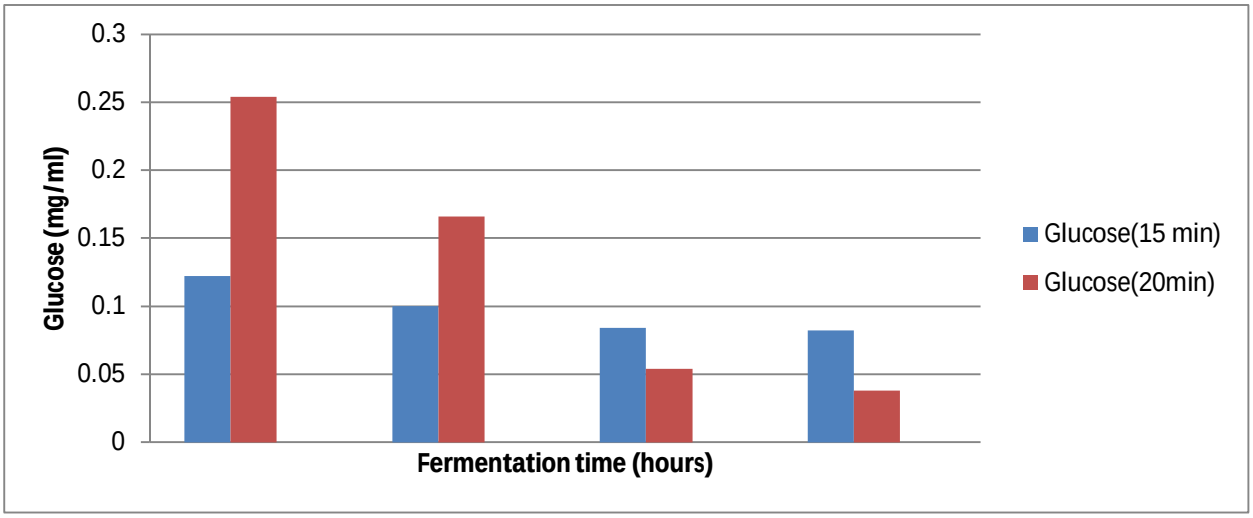


Figure 9: Variation in Glucose concentration during fermentation of pasteurized pomegranate juice by *L. plantarum*

J. Variation in Microbial population of pomegranate juice during fermentation by L. plantarum

Changes in viable cell counts during pomegranate juice fermented by *L. plantarum* are given in Figure 9. Lactic acid culture grew rapidly in pasteurized pomegranate juice and reached a viable cell population 4.2 x10^7 in 15 min pasteurized juice and 3.9 x10^7in 20 min pasteurized juice sample after 48 hours fermentation at 30°C. The management of the fermentative process is important from several points of view, among which obtaining drinks with desirable sensory characteristics and existence of at least 10^7 CFU lactic acid bacteria /ml, which make the drink a probiotic product. Some authors were reported that acid production ability by lactic acid bacteria, especially post acidification, affected the cell viability of probiotic bacteria.

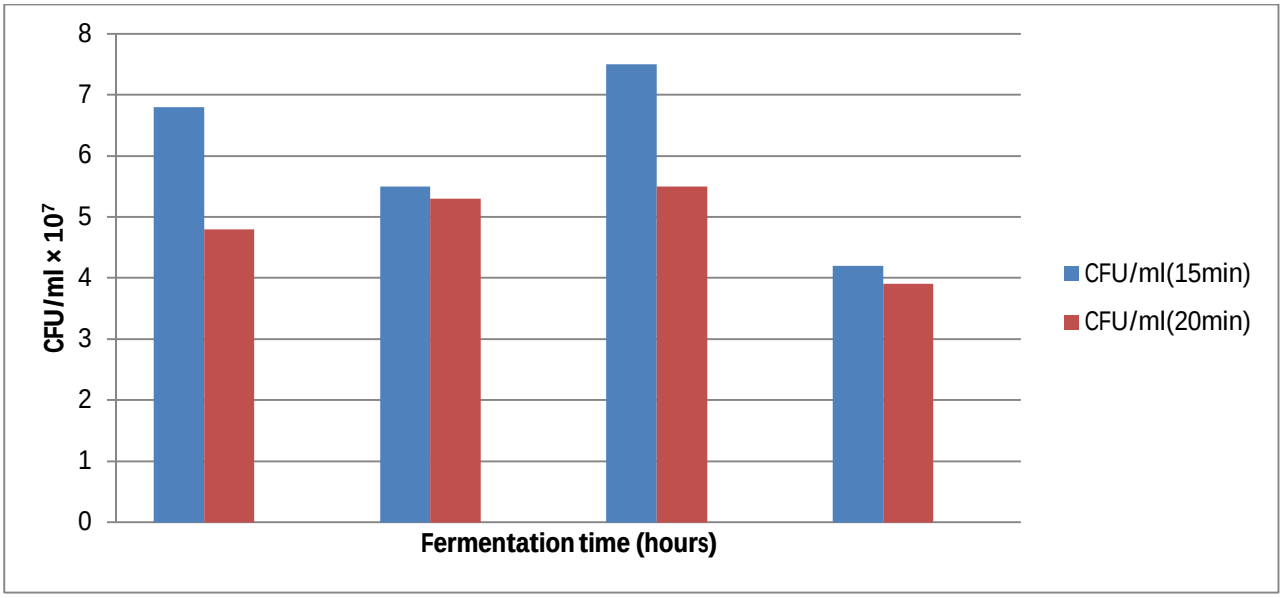


Figure 10: Growth of *L.plantarum* during fermentation at 30°C in pasteurized pomegranate juice

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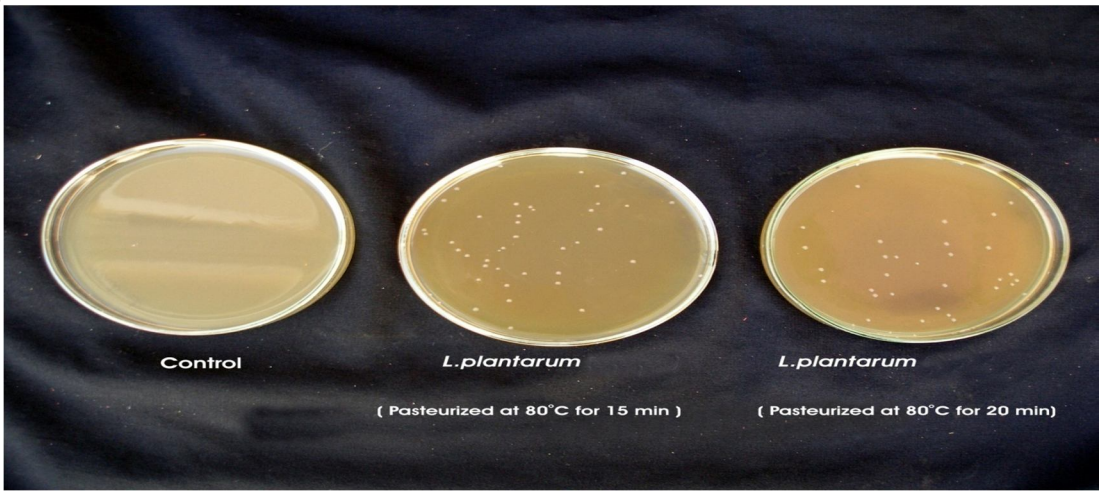


Plate No. 4.5 Growth of *L. plantarum* in pasteurized pomegranate juice during fermentation at 30°C for 0 hour

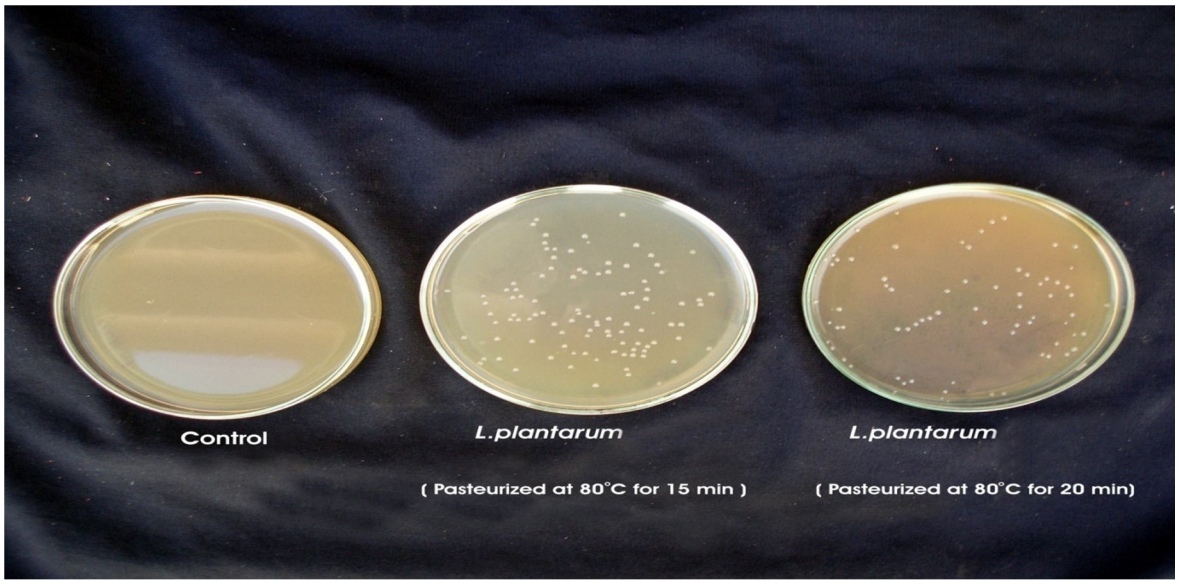


Plate No. 4.6 Growth of *L. plantarum* in pasteurized pomegranate juice during fermentation at 30°C for 24 hrs.



Plate No. 4.7 Growth of *L. plantarum* in pasteurized pomegranate juice during fermentation at 30°C for 48 hrs.

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K. Effect of cold storage(4°C) on the viabilityof lactic acid cultures in fermented probiotic pomegranate juice

Fermentation of pomegranate juice using Lactic acid cultures at 30°C for 72 hours provided the products containing higher than10^6 cfu/ml after storage for 3 week at 4°C. Conversely, It was reported that the probiotication provided the high number of the cells above the benefit level in fruit juices, the sensory characteristics of the product were not accepted by the consumers. The above pic. illustrates the effect of cold storage on the viability of *Lactobacillus acidophilus* in fermented juices.

Table 6: Effect of cold storage (4°C) on the viability of *L. acidophilus* in probiotic pomegranate juices

Time in Weeks	*L. acidophilus*	
	Probiotic pomegranate juice (Pasteurized at 80°C for 15 min)	Probiotic pomegranate juice (Pasteurized at 80°C for 20 min)
	Stored at 4°C	Stored at 4°C
	CFU/ml x10^7	CFU/ml x10^7
0	12.5	9.5
1	12.0	9.6
2	7.5	7.4
3	7.4	7.2
4	6.9	6.5

Due to L. acidophilus 15 Min: $F_{(cal)}$, = 397> $F_{(tab)}$ =4.76(5% and 4 degree of freedom)–Significant; CD= 0.499
Due to L. acidophilus 20 Min: $F_{(cal)}$, = 43.76> $F_{(tab)}$ =4.76(5% and 4 degree of freedom)–Significant; CD= 0.643

L. Survival of lactic acid bacteria in fermented pomegranate juice during cold storage

The *Lactobacillus* spp. was capable of surviving at low pH and high acidity condition in the fermented pomegranate juice. Figure 4.9 shows that the microbial population of *L. acidophilus* in 15 min pasteurized juice was decreased to 6.9 x10^7cfu/ml and 6.5 x10^7 cfu/ml in 20 min pasteurized juice even after 3 weeks at 4°C. *L. acidophilus* shows better viability in 15 min pasteurized juice than that grown in 20 min pasteurized juice. The reason could be addressed to the lack of their ability to survive in the stressful condition of low pH and high acidity of the pomegranate juice and also relatively low temperature of the environment (4°C). Similar results were obtained for fermented tomato juice by other authors (Yoon 2004).

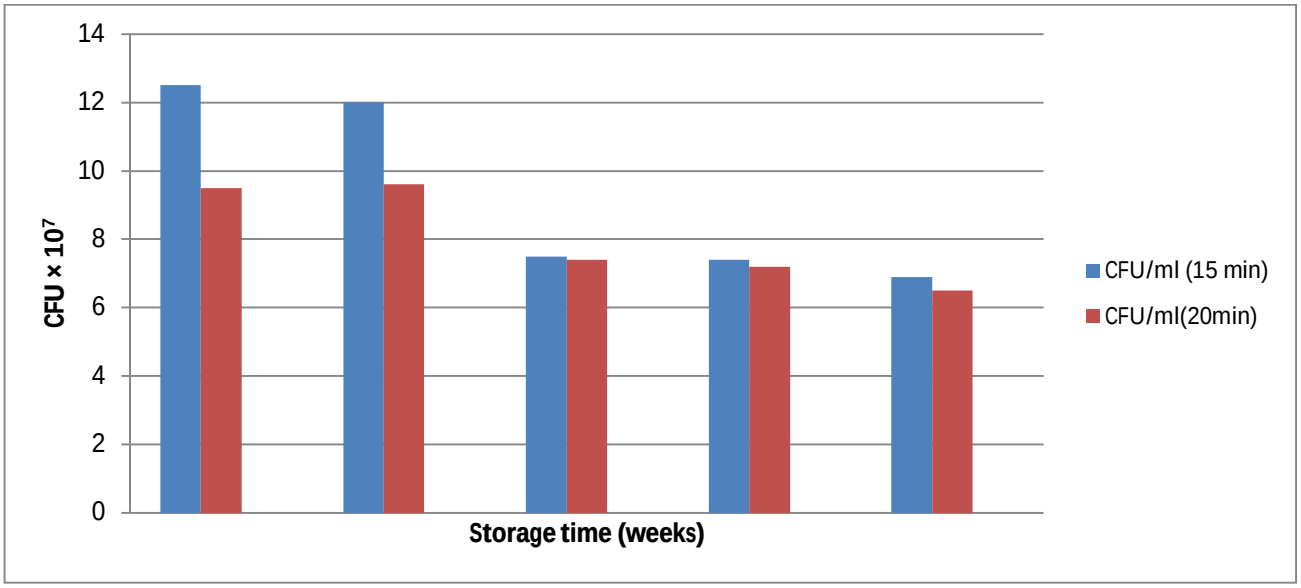


Figure 11: Effect of cold storage (4°C) on the viability of *L. acidophilus* in fermented pomegranate juice

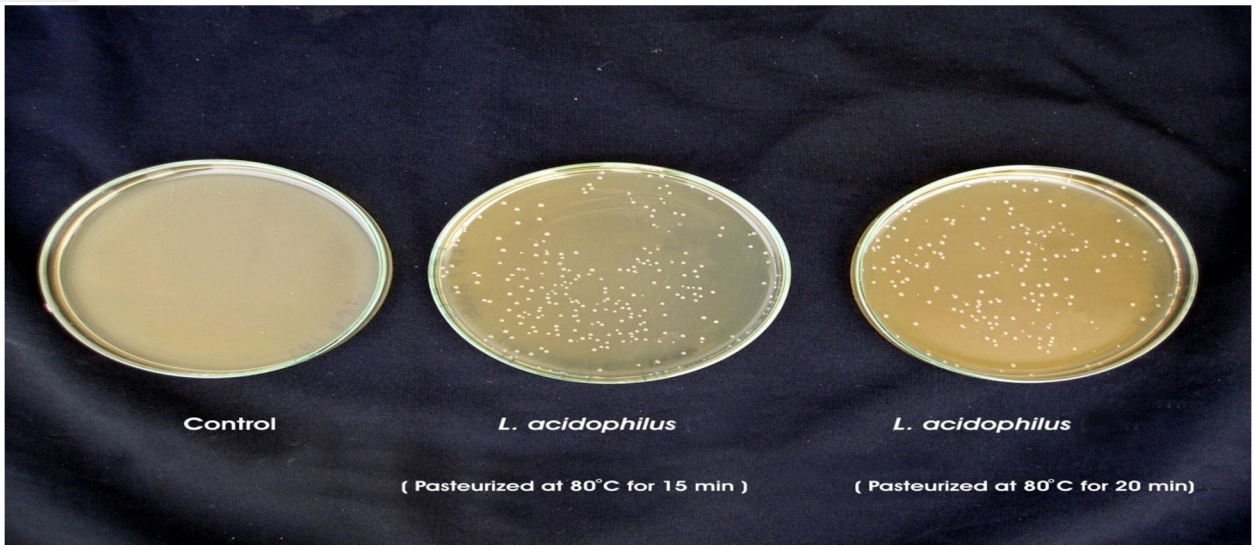


Plate No. 4.9 Viability of *L. acidophilus* in pasteurized pomegranate juice during storage at 4°C for 0 week

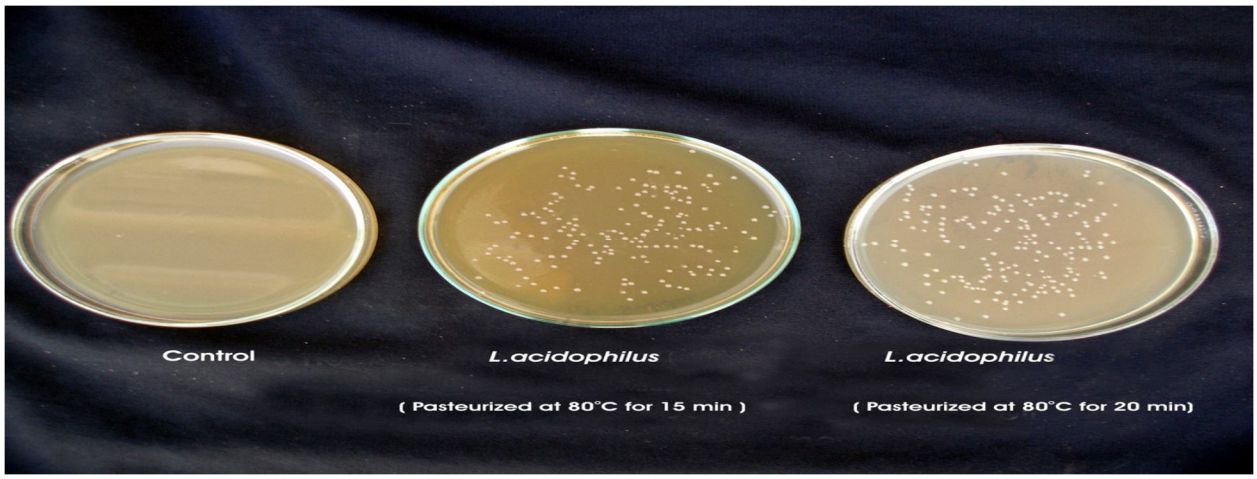


Plate No.4.10 Viability of *L. acidophilus* in pasteurized pomegranate juice during storage at 4°C for 1 week.

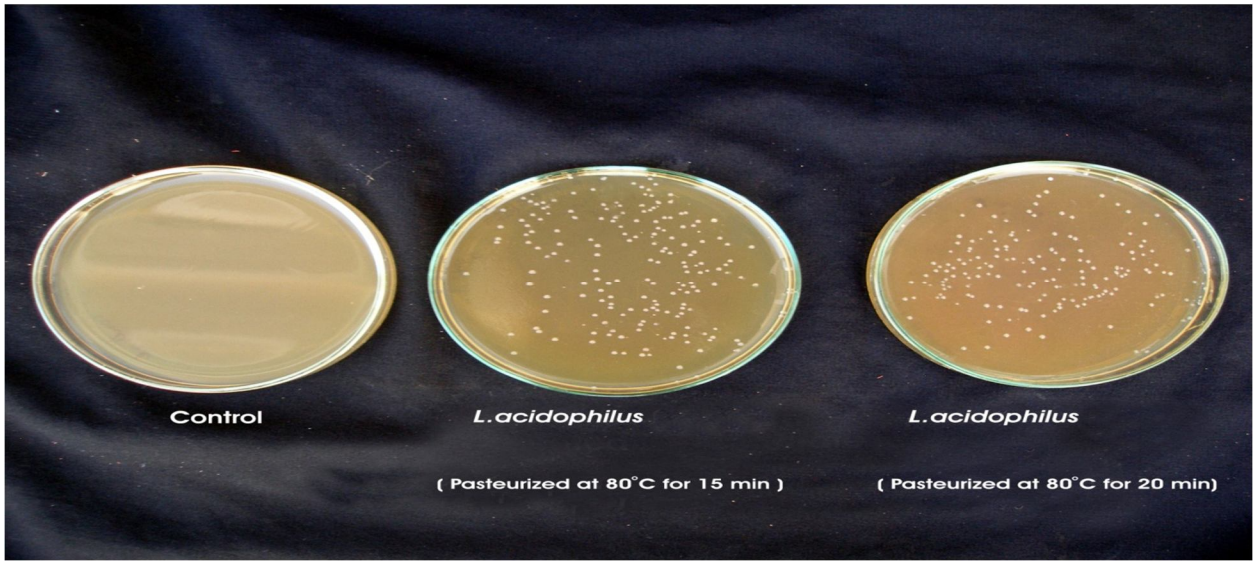


Plate No.4.11 Viability of *L. acidophilus* in pasteurized pomegranate juice during storage at 4°C for 2 weeks

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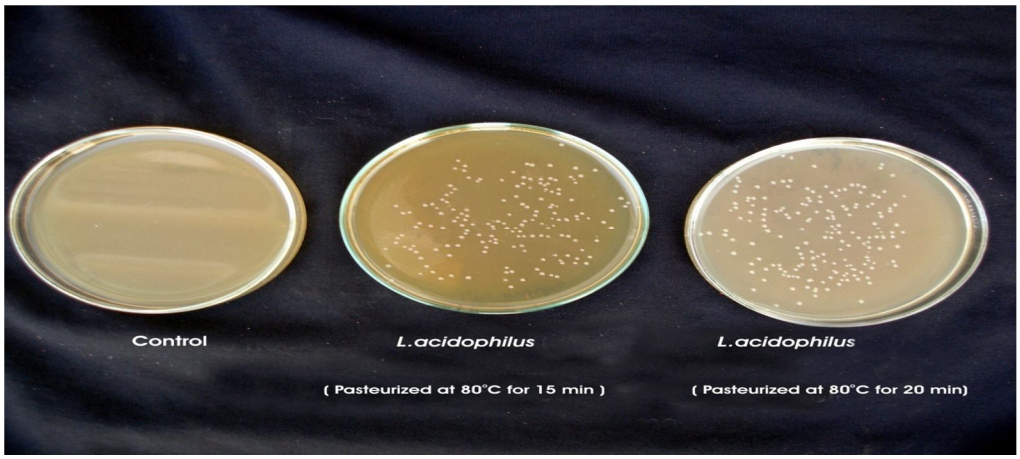


Plate No. 4.12 Viability of *L. acidophilus* in pasteurized pomegranate juice during storage at 4°C for 3 weeks

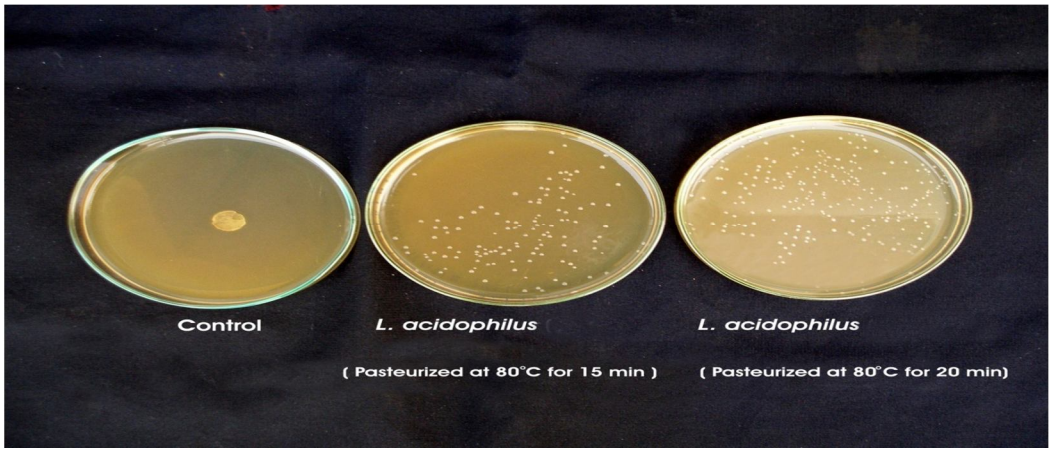


Plate No.4.13 Viability of *L. acidophilus* in pasteurized pomegranate juice during storage at 4°C for 4 weeks

M. Effect of cold storage(4°C) on the viabilityof lactic acid cultures in fermented probiotic pomegranate juice

The viable cell counts of *L. plantarum* decreased slightly during cold storage, but the cell viability of the *L. plantarum* remained at a considerably high level (10^7cfu/ml) after 3 weeks of cold storage. Table 4.13 illustrates the effect of cold storage on the viability of *Lactobacillus plantarum* in fermented juices.

Table 7 Effect of cold storage (4°C) on the viability of *L. plantarum* in probiotic pomegranate juices

Time in Weeks	*L. plantarum*	
	Probiotic pomegranate juice (Pasteurized at 80°C for 15 min)	Probiotic pomegranate juice (Pasteurized at 80°C for 20 min)
	Stored at 4°C	Stored at 4°C
	CFU/ml x 10^7	CFU/ml x10^7
0	4.2	3.9
1	5.5	4.5
2	4.5	4.0
3	4.2	3.5
4	3.3	2.5

Due to *L. plantarum* 15 min: $F_{(cal)}$, = 16.53> $F_{(tab)}$ =4.76(5% and 4 degree of freedom)–Significant; CD= 0.582

Due to *L. plantarum* 20 min: $F_{(cal)}$, = 12.45> $F_{(tab)}$ =4.76(5% and 4 degree of freedom)–Significant; CD= 0.0.637

N. The survival of lactic acid bacteria in fermented pomegranate juice during cold storage:

In Figure 12 *L. plantarum* shows better viability in15 min pasteurized juice than that grown in 20 min pasteurized juice. According to Shah (2001) it is important to have a significant number of viable lactic acid bacteria present in the probiotic products for maximum health benefits. According to Dave and Shah (1997) Lactic acid starters are reported to produce bacteriocin against probiotics bacteria and vice versa. In general, the cell viability depends on the strains used, interaction between species present, culture condition, oxygen content, and final acidity of the product and the concentration of lactic acid. The main factor for loss of viability of probiotic microorganism have been attributed to the decrease in pH of the medium and the accumulation of organic acid as a result of growth and fermentation (Hood and Zottola, 1998)

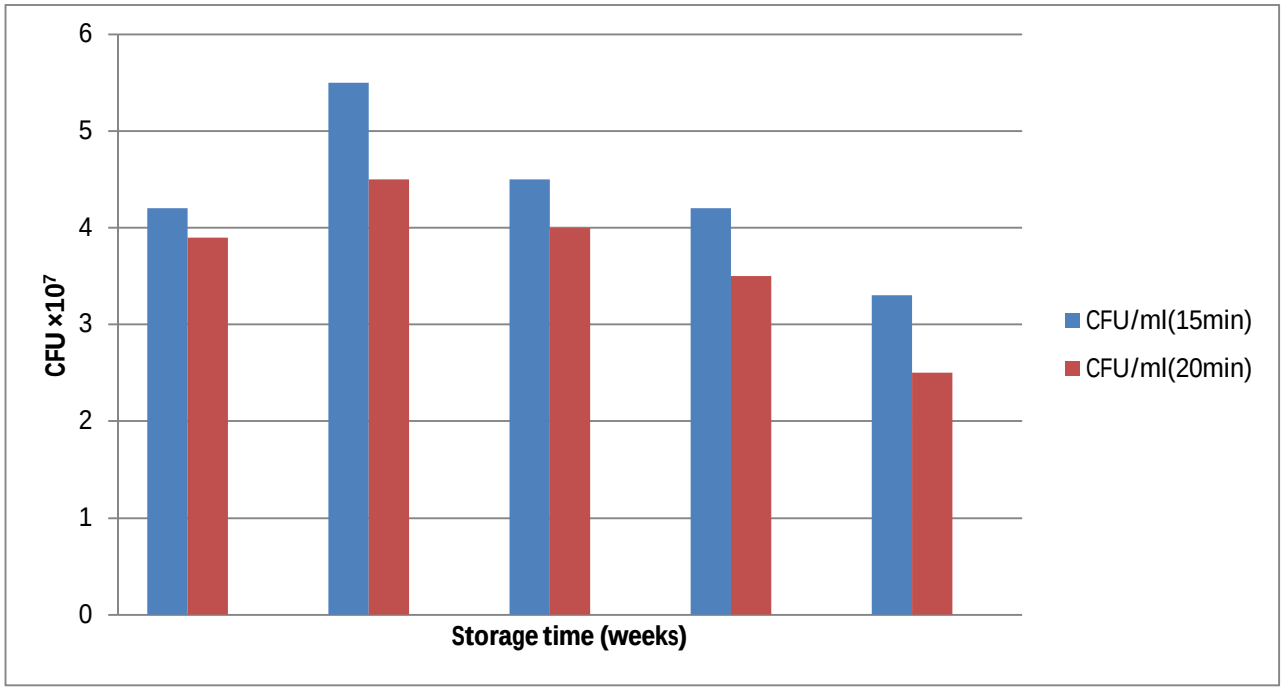


Figure :12 Effect of cold storage (4°C) on the viability of *L. plantarum* in fermented pomegranate juice



Plate No. 4.14 Viability of *L. plantarum* in pasteurized pomegranate juice during storage at 4°C for 0 week

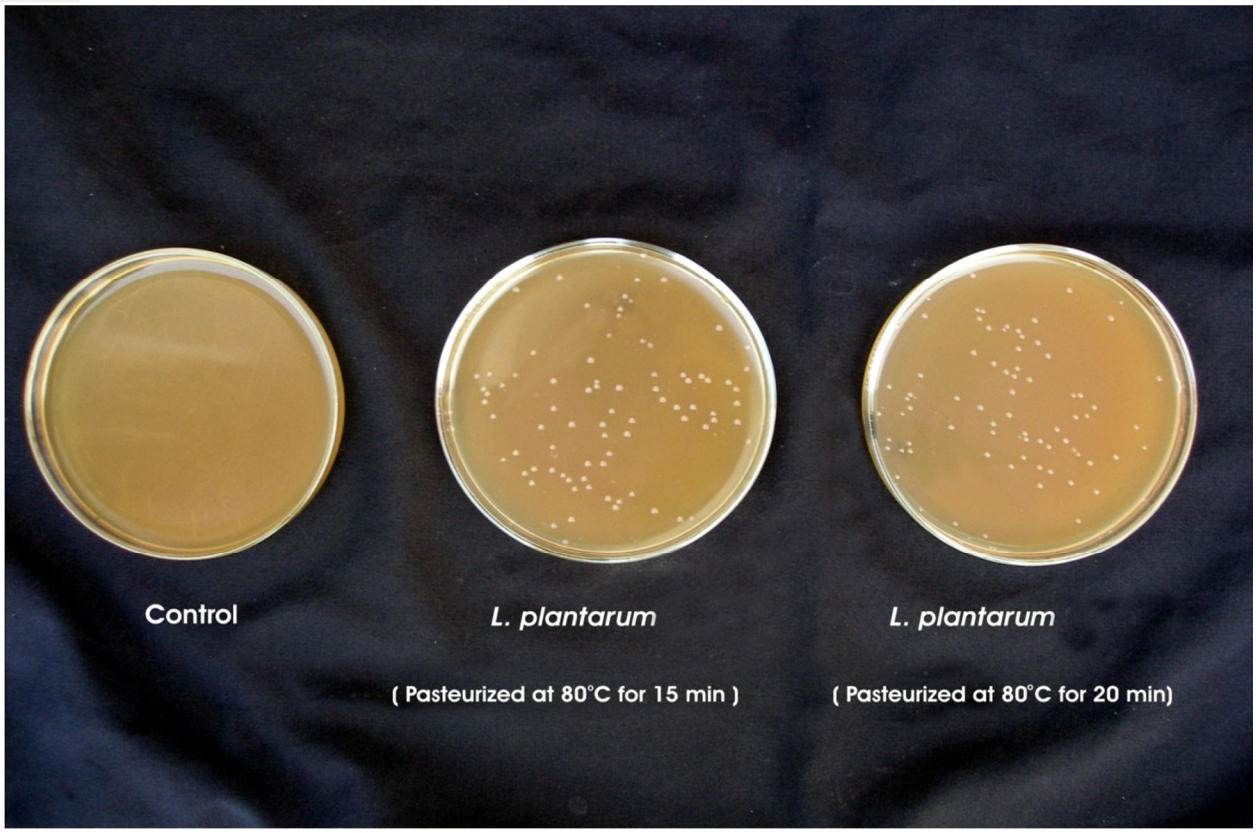


Plate No. 4.15 Viability of *L. plantarum* in pasteurized pomegranate juice during storage at 4°C for 1 week

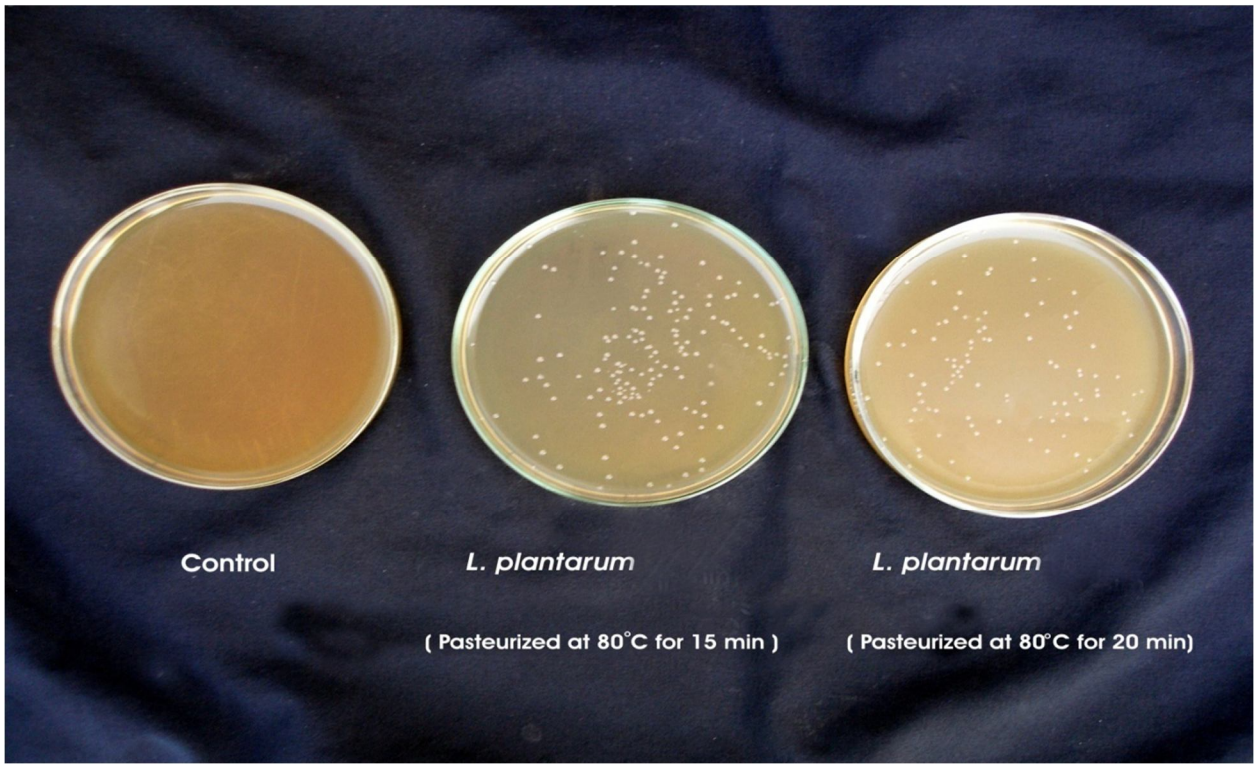


Plate No. 4.16 Viability of *L. plantarum* in pasteurized pomegranate juice during storage at 4°C for 2 weeks

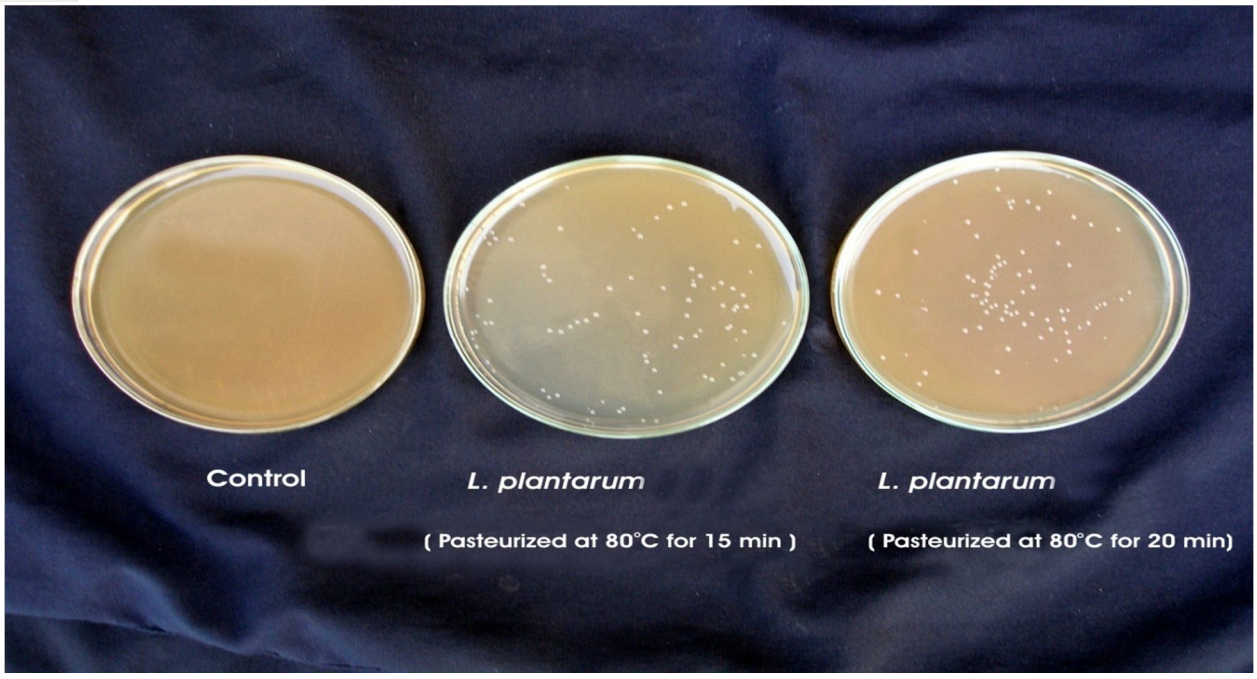


Plate No. 4.17 Viability of *L. plantarum* in pasteurized pomegranate juice during storage at 4°C for 3 weeks

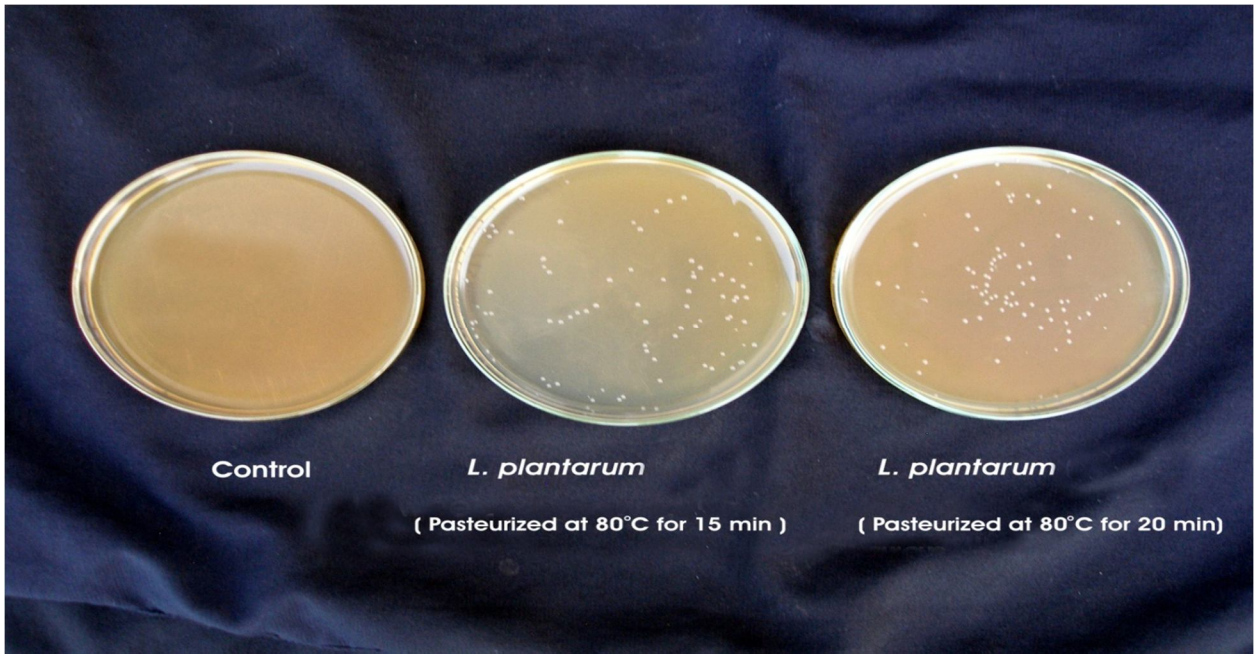


Plate No. 4.18 Viability of *L. plantarum* in pasteurized pomegranate juice during storage at 4°C for 4 weeks

IV. CONCLUSION

It is concluded that the fermented pomegranate juice could be used as raw material for lactic acid fermentation and the product could serve as a healthy beverages for consumers who are allergic to dairy product. Both *L. planatrum* and *L. acidophilus* are suitable for use as probiotics cultures for the production of probiotics fruit juices. During the fermentation process there is an accumulation of acid with higher rate in case of *L. acidophilus* which make the pomegranate juice much pleasant in terms of sensory trades. Fermented pomegranate juice could be recommended to be used for the preparation of food based product process by lactic acid fermentation.

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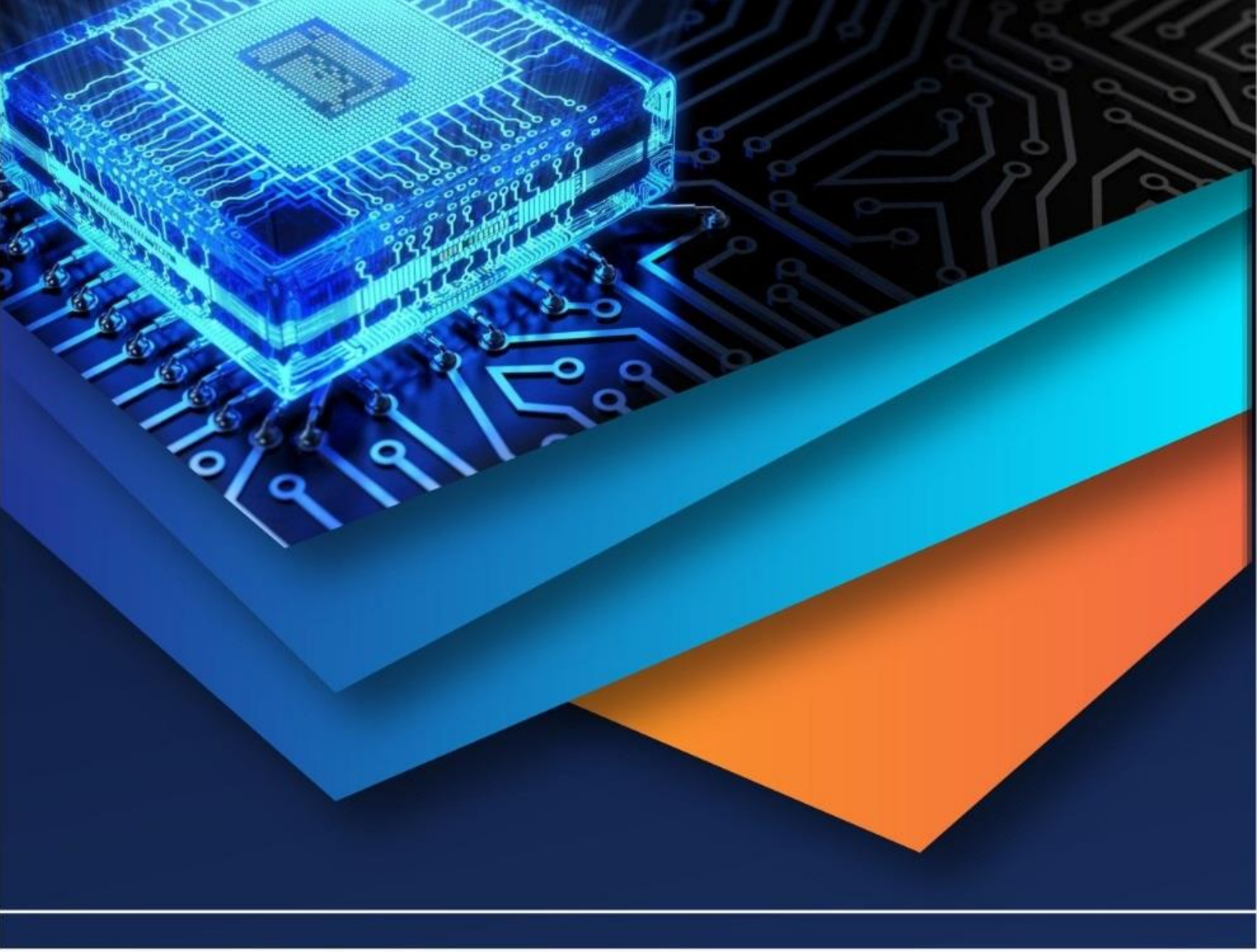
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