

Fortification and fermentation of non-dairy products with probiotic lactobacilli

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Surveys and studies on the non-dairy probiotic beverages and foods are developing continuously, the vegetables and fruit juices is showing a promising growth on the pro-biotics. In this study, the Fortification and fermentation of non-dairy products with probiotic lactobacilli has been performed. Fermentation of the fruit juices is a long process, by the application of water bath it has been performed to make it a quick.

Keywords: cholesterol, fiber chain, pH Analysis

1. Introduction

The food products are considered as pro-biotics when the range reaches 106CFU/mL of minimum value [1, 2]. A wide variety of strains of pro-biotics are available that are used in the food materials, but the most known belongs to the Bifidobacterium and Lactobacillus group [3]. The bile and acid resistant group survives the tracts of gastrointestinal. An effect of the urinary infection tract by applying the urogenital pathogen adhesion [4]. Another application of this strain is to create the probiotic yogurt in the eastern part of Africa. The probiotic yogurt has been widely spread in mostly the eastern Africa communities and mostly consumed by some HIV or AIDS patients [5].

Prebiotics go about as the pro-biotics fuel and are found normally in an assortment of food varieties like garlic, on leeks and leeks [6]. Prebiotics get characterized as "a specifically fermented materials that permits explicit changes, both in activity as well as composition in the microflora gastrointestinal, which provides a list of benefits in health [7]. Prebiotics get displayed to upgrade the practicality of pro-biotics and work on gastrointestinal wellbeing and upgrade calcium ingestion [6]. Inulin fiber is a kind of prebiotic that is a lowcalorie sugar and fat substitute that goes about as a building specialist and advances a solid stomach microflora [8,9]. The Short fiber chain inulin has been displayed to raise the L. rhamnose us viability more than the long inulin chain when added to yogurt [8]. Since numerous North Americans are neglecting to meet the RDA for every day fiber [7], the fortress of inulin fiber to probiotic organic product juices will empower purchasers to improve their fiber admission. Epidemiologic studies recommend that satisfactory fiber admission is related with a decrease in cardiovascular illness results and coronary illness, basically by bringing down LDL cholesterol levels [7].

The idea of the food transporter is likewise significant for the endurance of bacterial strains during their entry through the gastro-digestive system [11]. Specialists observed that the protein from nut flour aged by Lactobacillus plantarum P9 can upgrade the endurance of probiotic cells in mimicked gastric and bile juices. Actual security of pro-biotics by microencapsulation is one of the most-involved approaches for development of probiotic endurance during capacity and after utilization. The most well-known strategies for

microencapsulation of pro-biotics incorporate embodiment by expulsion, emulsification, shower drying and freeze drying, while alginate, chitin, gelatin, k-carrageenan are the most normally utilized transporters [12]. Immobilization of pro-biotics for the assembling of drinks is normally utilized by numerous scientists to accomplish high cell thickness maturations for both cell and metabolite creation. The size of the microcapsules is significant, in light of the fact that it impacts the mass exchange between the cell and the encompassing medium, as well as the tangible properties of food varieties. To be specific, enormous gel dabs can weaken mouth-feel properties of a liquid item. The widths of the subsequent gel dots framed by the expulsion still up in the air by the spout breadth and liquid thickness, and can differ from 1.5 mm [13] to 3 mm [14]. Microencapsulation by emulsification and splash drying produces dabs with a lot more modest width (0.1-1.0 mm), however these strategies are more costly than the basic expulsion technique.

After a continuous review on publications on the pro-biotics efficacy of the fruit juices, so far no trace of *L. rhamnosus* GR-1 has been found ad strain. The target of this study is to decide and analyse the *L. rhamnosus* potential in fruits like grape, orange that are protected with the inulin fiber for a period of 30 days and to detect the acceptability of the consumers by performing a sensory evaluation.

2. Materials

Preparation of Probiotic Solution:

Sterilization in the DeMan, Sharpe and Rogosa, the broth of MRS with the *L. rhamnosus* GR-1, later gas pack incubation at a temperature of 37°C for a period of 24 hrs has been performed. The solution then refrigerated at a temperature of 4°C, this process continued every week to get a continuous *L. rhamnosus* GR-1 viability.

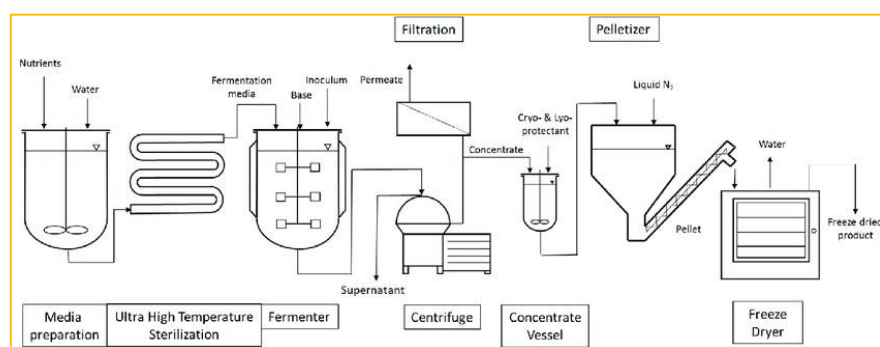


Figure 1: Schematic representation of the of pro-biotics production for dairy culture

Preparation of Fruit Juice:

To perform this 100% natural fruit juices are selected that are available in the commercial market that contains no sugar, or preservatives, or any kind of colours. The detailed has been discussed below

Fermentation of Fruit Juice:

Fermentation of the fruit juices is a long process, by the application of water bath, total seven beakers that contains 80mL of every variety of juices left to be heated until it reaches 85°C, that needs to be maintained for 10 min, later the temperature dropped down to 37°C and *L. rhamnosus* GR-1 has been added. After mixing the solution properly it need to be settle down. Each of the beaker has been allotted a specific time of storage and fermentation.

Beaker 1 allotted 0 time, whereas the rest beakers ranged from 24 to 72 h. the beaker number 2 to 7 placed with an anaerobic gas pack container and placed for incubation at a temperature of 37°C. Now, the beaker

number 2 has been removed after 24h of the process, the beaker 3 removed after 48h and the rest removed after 72h. The beaker from 5 to 7 refrigerated at 4°C, and kept for 10 days, 20 days and 30 days respectively.

Incorporation of long and short inulin fiber chain with fruit Juice:

During the water bath process, 4% of the long and short inulin fiber chain has been added in the respective juices in the 80mL of every variety of juices left to be heated until it reaches 85°C, that needs to be maintained for 10 min, later the temperature dropped down to 37°C and *L. rhamnosus* GR-1 has been added. After mixing the solution properly it need to be settle down. Each of the beaker has been allotted a specific time of storage and fermentation.

Beaker 1 allotted 0 time, whereas the rest beakers ranged from 24 to 72 h. the beaker number 2 to 7 placed with an anaerobic gas pack container and placed for incubation at a temperature of 37°C. Now, the beaker number 2 has been removed after 24h of the process, the beaker 3 removed after 48h and the rest removed after 72h. The beaker from 5 to 7 refrigerated at 4°C, and kept for 10 days, 20 days and 30 days respectively.

3. Methods

Analysis of Microbial

Count of *L. rhamnosus* GR-1 from all natural product juice tests was completed for fermentation seasons of 0h, 24h, 48h, and 72h and during the period of storage of consecutive 1, 10, 20, 30 days. Subsequent plating and Serial dilutions utilized to decide the counts of microbial of the *L. rhamnosus* GR-1. From the organic product juice tests, dilution of 1.01mL aliquots in clean 0.86% solution of saline at the separate factors of dilution. Utilizing a pipette, 0.1 mL of each organic juice test was plated. Utilizing 5.23%(w/v) MRS, 1.6% (w/v) agar and 0.0016% (w/v) fusidic corrosive Agar plates prepared.

pH Analysis

After an evaluation of the fruit sample fermentation process for 0h, 24h, 48h and 72h and after a respective time gap duration. Two replicas has been collected at each storage and fermentation process.

Statistical Analysis

Statistical analysis has been performed using the IBM SPSS analysis software. By the application of ANOVA test the analyzation of of *L. rhamnosus* GR-1 viability has been performed. With the use of one way ANOVA and the LSD to means comparisons of the data has been performed.

4. Results

Analysis of microbial

The count of *L. rhamnosus* GR-1 viable reached around 106.5CFU/mL for all the samples of juice during the process of fermentation. The table shows the counts of the microbial over the fermentation time period. The no-significant represents $p > 0.05$, however, it shows a significant difference i.e between the time of fermentation and the juice. After a fermentation of over 24h the OC sample average count is 7.34 ± 7.23 that is significantly much higher than AC that has an average count of 2.71 ± 1.85 .

Table 1: (Mean $\pm$ SD) Viable counts of *L. rhamnosus* GR-1

Treatment	0h	24h	48h	72h	The p-Value
AC	4.05 ± 3.89	2.71 ± 1.85	8.49 ± 7.23	3.01 ± 2.27	More than 0.05
ALC	5.80 ± 4.71	1.14 ± 0.86	3.91 ± 2.31	7.50 ± 5.18	More than 0.05
ASC	1.92 ± 0.81	2.32 ± 1.94	3.42 ± 1.68	1.63 ± 0.47	More than 0.05

GC	1.02 ± 0.26	1.71 ± 1.50	2.63 ± 2.10	2.34 ± 1.66	More than 0.05
GLC	0.63 ± 0.12	4.23 ± 1.01	0.25 ± 0.16	0.56 ± 0.38	More than 0.05
GSC	1.05 ± 0.12	1.94 ± 1.72	0.30 ± 0.03	1.79 ± 1.73	More than 0.05
OC	1.02 ± 0.24	7.34 ± 7.23	13.2 ± 4.02	6.47 ± 0.82	More than 0.05
OLC	2.82 ± 2.01	6.65 ± 2.71	5.56 ± 1.24	5.96 ± 4.60	More than 0.05
OSC	2.21 ± 1.71	4.47 ± 3.61	6.55 ± 1.21	5.62 ± 1.61	More than 0.05

pH Analysis

The obtained results from the fermented juice samples by *L. rhamnosus* GR-1 performed in the collected pH initial values that are presented in the Figure 1. In all the mentioned cases (5.1, 4.2 and 3.5) the process of fermentation initiated from the common count, i.e 107 CFU/mL. According to the report, 3.5 pH value case, not supports the culture growth, though the pH modified values showed. The high intensity growth shows in the 5.1 pH value case, that leads to the 1.3×10^{10} CFU/mL within 11h of the fermentation. In this case the growth of the culture shows an increment rather than decreasing.

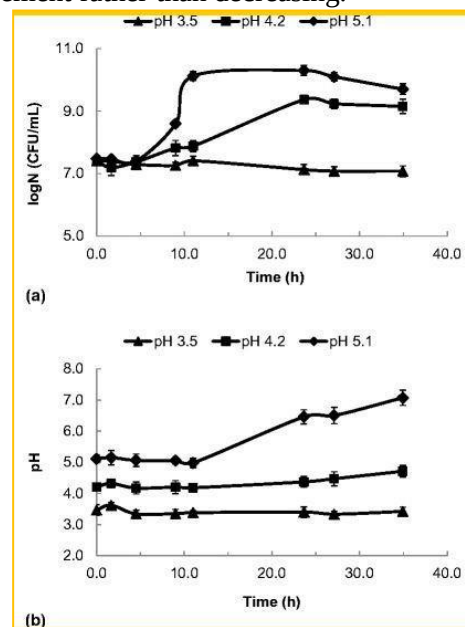


Figure 2: (a) viable-count of bacterial growth (a) and (b) pH-value count during the sample juice fermentation by *L. rhamnosus* GR-1

Statistical Analysis

The reported statistical curves of the growths are the outcomes of the fermentation process where an average of the data of the triplicate counts of the juice samples has been collected. The standard deviations represented as the error bars. However, for the kinetics of the microbial, 3-static models of growth fitted in the colony data count, i.e the shifted log function (Equation 1), Gompertz modified model (Equation 2), and the logistic modified function [10]. Akaike Criterion determined the goodness fit for the first time that represents the values that was used to detect the kinetic parameters.

Equation 1: Shifted logistic function

$$Y(t) = Y_{As} \left[\frac{1}{1 + \exp(k(t_c - t))} - \frac{1}{1 + \exp(kt_c)} \right]$$

k= specific rate of growth,

t c = lag phase time

Equation 2: Gompertz modified model

$$\ln \frac{N}{N_0} = A_s \exp \left[-\exp \left(\frac{\mu_{max} e}{A_s} (\lambda - t) + 1 \right) \right]$$

Equation 3: th logistic emodified function

$$\ln \frac{N}{N_0} = \frac{A_s}{1 + \exp \left[\frac{4\mu_{max}}{A_s} (\lambda - t) + 2 \right]}$$

The parameters in these two equations 2, 3, represents the microbial re-parameterized interpretable parameters.

N represents the count of colony at t

N₀ represents the count of colony at t=0

5. Conclusion

The bacterium *L. rhamnosus* GR-1 applied to initiate the fermentation of the probiotic beverages. The juices yields 5% v/v as the best fermentation results in perspective of the high growth rate and maximum cell concentration. The no-significant represents $p > 0.05$, however, it shows a significant difference i.e between the time of fermentation and the juice. After a fermentation of over 24h the OC sample average count is 7.34 ± 7.23 that is significantly much higher than AC that has an average count of 2.71 ± 1.85 . The statistical analysis application shows a justification on the tests by using the variety of equations. As a future scope, a sensory assessment of the samples can be done.

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