The Pharma Innovation Journal 2021; 10(11): 1842-1845

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 1842-1845

www.thepharmajournal.com
Received: 13-08-2021
Accepted: 21-10-2021

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Effect of prebiotics on growth and survival of *Lactobacillus acidophilus* (LA 5), *Bifidobacterium bifidum* (BB 12) in cheddar cheese during storage

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Abstract
The aim of this study was to produce a synbiotic cheddar cheese with pectin, inulin and maltodextrin as prebiotics at different levels namely, 2.0%, 1.5% and 2.5% respectively and how these prebiotics influence the growth and survivability of the probiotic microorganisms *viz. Lactobacillus acidophilus* (LA 5) and *Bifidobacterium bifidum* (BB 12).
Various combination of prebiotics were also tried to evaluate the survivability of *Lactobacillus acidophilus* (LA 5) and *Bifidobacterium bifidum* (BB 12) under refrigerated storage (4 ± 1 °C) for 120 days. It was shown that the probiotic count in log cfu/g markedly increased up to 60 days. After 60 days,a gradual decline in microbial count was observed. It was concluded that pectin (2.0%), inulin (1.5%) and maltodextrin (2.5%) combination was found to give good results in enhancing the probiotic bacterial count for production of synbiotic cheese.

Keywords: Cheddar cheese, probiotics, survivability, prebiotics, pectin, inulin, maltodextrin

Introduction
Probiotics are live bio-therapeutic agents that can serve as an alternative to antibiotic therapy, especially with regard to the development of resistant populations of pathogenic bacteria (Oyetayo *et al*, 2005) [11]. Most human probiotic species are members of two genera of lactic acid bacteria, *Lactobacillus* and *Bifidobacterium* (Quinto *et al*, 2014) [13]. In recent years, fermented milk, yoghurt and cheese have been used as a popular vehicle for delivering probiotic bacteria in food (Ashraf and Shah, 2011) [1]. Cheeses are gaining more reputation in the world due to their nutritional value, variety in texture and flavour, functionality and its convenient uses (Farkye, 2004) [4]. Cheddar is highly nourishing and is rich in fat, protein, vitamins and minerals (Murtaza, 2016) [9]. Prebiotics were first defined as food ingredients that are non digestible but beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, helps in improving host health (Mali *et al*, 2020) [7]. The oligosaccharides obtained from pectin have been proposed as an excellent candidate for new-generation prebiotics (Mandalari *et al*, 2007) [8]. Fructo oligosaccharides (FOS) and inulin have attracted much attention lately as prebiotics. FOS contains 2–10 fructose units linked by glycosidic bonds, while inulin includes a broad range of fructans with chains of 3–60 units (Rossi *et al*, 2005) [14]. Another oligosaccharide with prebiotic properties is maltodextrin. Maltodextrins are malto oligosaccharides with a degree of polymerisation ranging from 3 to 9 and often act as fat replacers and bulking agents in foods (Oliveira *et al*, 2009) [10]. The objective of the present study is to evaluate the effect of prebiotics such as pectin, inulin and maltodextrin on the growth and viability of *Lactobacillus acidophilus* (LA 5) and *Bifidobacterium bifidum* (BB 12).

Material and Methods
Procurement of Material
Pasteurized standardized milk (AAVIN) was purchased from local market of Koduveli, Chennai, Tamil Nadu for the manufacture of cheddar cheese. Freeze dried mesophilic culture (Chr. Hansen's) was used for cheese acidification. *Lactobacillus acidophilus* (LA5), *Bifidobacterium bifidum* (BB12) were purchased from Chr. Hansen, Bengaluru, Karnataka and used for production of probiotic cheddar cheese. Microbial rennet Fromase® in tablet form was utilized at the rate of 0.01% for cheddar cheese preparation.

Pectin ($C_7H_2OO_6$ - 130-260), Inulin ($(C_6H_{10}O_5)x$), and Maltodextrin ($C_6nH(10n+2)O(5n+1)$) in powder form were used as prebiotic for cheddar cheese preparation.

Cheddar cheese making protocol

Cheddar cheese was produced with minor modifications as per the method as followed by Scott (1981) [15]. Milk was heated to 63°C for 30 minutes. It was cooled to 30-31°C and added with 1.0 per cent starter culture for acid production. After 30 minutes, renneting was done by addition of microbial rennet at the rate of 0.01 g for 1 litre of pasteurized milk. The milk was allowed to coagulate (setting) for 45 minutes. Then the curd was cut with cheese knives. The curd mass was cooked to 38-39 °C for 45 minutes with slow agitation. The whey was drained out and cheddaring was done at 39 °C. The curd mass was turned every 15 minutes until the acidity increased to 0.45 to 0.50% lactic acid followed by milling of curd mass. Selected levels of probiotics and prebiotics were added with that of milled curd and salting (1%) was done. It was hooped in cheese moulds, dressed and pressed overnight. The cheese blocks were turned after 8 hours and pressed for 24 hours. After pressing, cheese blocks were removed from the press and kept in refrigerator for 2 days for surface drying and then packed and stored at 4 ± 1°C.

Statistical analysis

The data were subjected to statistical analysis with SPSS (version 20.0) software as per the standard procedure outlined by Snedecor and Cochran (1995).

Results and Discussion

The mean ± SE values of *Lactobacillus acidophilus* count (Log_{10}cfu/g) of cheddar cheese incorporated with different combinations of pectin, inulin and maltodextrin (T1- *L. acidophilus* + Pectin, T2- *L. acidophilus*+ Inulin, T3 - *L. acidophilus* + Maltodextrin, T4 - *L. acidophilus*+ Pectin + Inulin, T5 - *L. acidophilus* + Pectin + Maltodextrin, T6 - *L. acidophilus* + Inulin + Maltodextrin and T7 - *L. acidophilus*+ Pectin + Inulin + Maltodextrin and cheddar cheese with *Lactobacillus acidophilus* but without prebiotic is taken as control) are presented in Table 1.

The Total Viable Count (TVC) expressed as Log_{10}cfu/g of *L. acidophilus* of control cheddar cheese varied between 7.69±0.01 and 8.18±0.01 during different storage periods up to 120 days when stored at refrigerated temperature under aerobic packaging. The TVC for T1 varied from7.70±0.01 to 8.33±0.02; T2 from 7.70±0.02 to 8.31±0.01; T3 from 7.70±0.01 to 8.32±0.01; T4 from 7.69±0.01 to 8.33±0.01; T5from 7.71±0.01 to 8.34±0.01; T6from 7.70±0.01 to 8.33±0.01 and T7 between 7.70±0.01 and 8.49±0.01during the 120 days of storage.

There was no significant (p>0.05) difference in the initial total viable count (Log_{10}cfu/g) of *Lactobacillus acidophilus* (LA 5) betweenT1, T2, T3, T4, T5, T6, T7 and control. However at the end of 60 days there was significant increase in the count of *Lactobacillus acidophilus* (LA 5) in T1, T2, T3, T4, T5, T6, T7 and control followed by rapid decline. The main factors for the loss of probiotic viability could be due to decrease in the pH of the medium and the accumulation of organic acids resulting from growth and fermentation (Shah, 2000) [16]. The count was highest in T7 with 8.49±0.01 Log_{10} cfu/g and lowest in control (7.96±0.01 Log_{10} cfu/g) at 120 days. This might be due to action of pectin, inulin and maltodextrin as growth promoter for L.acidophilus count in Cheddar cheese. The results concur with the findings of Yeo and Liong, 2010 who found that supplementation with maltodextrin and pectin increased the growth of probiotics in soy milk.

Table 1: Mean ± SE values of Total Viable Count of *Lactobacillus acidophilus* (LA 5) in cheddar cheese incorporated with prebiotics at refrigerated (4±1°C) storage (n=6)

Treatments	Days	Control	T1	T2	T3	T4	T5	T6	T7	F-Value
L. acidophilus count CFU (Log10)cfu/g	0	$7.69±0.01^{A}$	$7.70±0.01^{A}$	$7.70±0.02^{A}$	$7.70±0.01^{A}$	$7.69±0.01^{A}$	$7.71±0.01^{A}$	$7.70±0.01^{A}$	$7.70±0.01^{A}$	0.164^{NS}
	30	$7.84±0.01^{B}$	$7.86±0.03^{B}$	$7.84±0.01^{B}$	$7.85±0.01^{B}$	$7.85±0.00^{B}$	$7.85±0.01^{B}$	$7.85±0.01^{B}$	$7.85±0.01^{B}$	0.189^{NS}
	60	$8.18±0.01^{aE}$	$8.33±0.02^{bC}$	$8.31±0.01^{bC}$	$8.32±0.01^{bC}$	$8.33±0.01^{bC}$	$8.34±0.01^{bC}$	$8.33±0.01^{bC}$	$8.49±0.01^{cC}$	29.207**
	90	$7.90±0.01^{aC}$	$8.32±0.01^{bC}$	$8.29±0.01^{bC}$	$8.32±0.01^{bC}$	$8.32±0.01^{bC}$	$8.33±0.01^{bC}$	$8.31±0.01^{bC}$	$8.48±0.01^{cC}$	162.984**
	120	$7.96±0.01^{aD}$	$8.30±0.01^{bC}$	$8.28±0.01^{bC}$	$8.31±0.01^{bC}$	$8.29±0.01^{bC}$	$8.32±0.01^{bC}$	$8.30±0.01^{bC}$	$8.47±0.02^{cC}$	80.900**
F-Value		215.02**	205.48**	345.57**	455.91**	477.59**	324.90**	426.67**	530.99**	

C - Control cheddar cheese with *Lactobacillus acidophilus* (LA 5)
T1 - 2% Pectin incorporated cheddar cheese with *Lactobacillus acidophilus* (LA 5)
T2 - 1.5% Inulin incorporated cheddar cheese with *Lactobacillus acidophilus* (LA 5)
T3 - 2.5% Maltodextrin incorporated cheddar cheese with *Lactobacillus acidophilus* (LA 5)
T4 –2% Pectin + 1.5% Inulin with *Lactobacillus acidophilus* (LA 5)
T5 - 2% Pectin + 2.5% Maltodextrin with *Lactobacillus acidophilus* (LA 5)
T6 - 1.5% Inulin + 2.5% Maltodextrin with *Lactobacillus acidophilus* (LA 5)
T7 - 2% Pectin+ 1.5% Inulin+ 2.5% Maltodextrin with *Lactobacillus acidophilus* (LA 5)
Means bearing different superscripts within rows (a, b, c, d) differ significantly ($P < 0.05$) (NS- $P > 0.05$; *- $P < 0.05$; **- $P < 0.01$)
Means bearing different superscripts within columns (A, B, C, D) differ significantly ($P < 0.05$) (NS- $P > 0.05$; *- $P < 0.05$; **- $P < 0.01$)

The mean ± SE values (Log_{10}cfu/g) of *Bifidobacterium bifidum* in cheddar cheese incorporated with different combinations of pectin, inulin and maltodextrin (T1- *B. bifidum* + Pectin, T2- *B. bifidum* + Inulin, T3 - *B. bifidum* + Maltodextrin, T4 - *B. bifidum* + Pectin + Inulin, T5 - *B. bifidum* + Pectin + Maltodextrin, T6 - *B. bifidum* + Inulin + Maltodextrin and T7 - *B. bifidum* + Pectin + Inulin + Maltodextrin and Control (cheddar cheese with *B. bifidum* but without prebiotics) are presented in Table 2.

The viable count (Log_{10}cfu/g) of *B. bifidum* of control cheddar cheese ranged between 7.70±0.01 and 7.84±0.01; T1 between 7.70±0.01 and 8.39±0.01; T2 from 7.70±0.01 to 8.38±0.02; T3 between 7.70±0.02 and 8.41±0.02; T4 from 7.70±0.02 to 8.39±0.02; T5 between 7.70±0.01 and 8.44±0.02; T6 from 7.69±0.02 to 8.41±0.01 and T7 varied from 7.70±0.01 to 8.47±0.01 during the 120 days of storage period.

There was no significant (p>0.05) difference with respect to the initial count (Log_{10}cfu/g) of *B. bifidum* (BB 12) between T1, T2, T3, T4, T5, T6, T7 and control. At the end of 60 days of storage, highest count (Log_{10}cfu/g) in *B. bifidum* (BB 12) was observed in Control, T1, T2, T3, T4, T5, T6 and T7. Among the treatments, the maximum count (Log_{10}cfu/g) was observed in T7 (8.47±0.01) and minimum count (Log_{10}cfu/g) in control (7.75±0.01) at 120 days. The viable count of *B. bifidum* in Cheddar cheese was similar to the findings of Ozer *et al.*, (2005) [12] who found the counts of *B. Bifidum* increasing in yoghurt during storage. Goderska *et al.*, (2008) [5] opined that citrus pectin was shown to possess prebiotic functions, such as oligo galacturonide obtained from apple pectin which has also demonstrated the ability to stimulate the growth of Bifidobacterium. Maltodextrin showed the best results in increasing bifidobacterial count in full fat and skimmed synbiotic fermented dairy product (Bisar *et al*. 2015) [2].

Table 2: Mean ± SE values of Total Viable Count of *Bifidobacterium bifidum* (BB 12) in cheddar cheese incorporated with prebiotics at refrigerated (4±1 °C) storage (n=6)

Parameters	Treatments	Control	T1	T2	T3	T4	T5	T6	T7	F value
B. bifidum count (Log_{10}cfu/g)	0	$7.70±0.01^{A}$	$7.70±0.01^{A}$	$7.70±0.01^{A}$	$7.70±0.02^{A}$	$7.70±0.02^{A}$	$7.70±0.01^{A}$	$7.69±0.02^{A}$	$7.70±0.01^{A}$	0.05^{NS}
	30	$7.74±0.02^{aAB}$	$7.82±0.04^{bB}$	$7.81±0.01^{bB}$	$8.30±0.01^{cB}$	$8.35±0.01^{cdB}$	$8.36±0.02^{cdB}$	$8.35±0.01^{cdB}$	$8.38±0.01^{dB}$	249.80**
	60	$7.84±0.01^{aC}$	$8.39±0.01^{bC}$	$8.38±0.02^{bD}$	$8.41±0.02^{bcC}$	$8.39±0.02^{bB}$	$8.44±0.02^{cdC}$	$8.41±0.01^{bcC}$	$8.47±0.01^{dC}$	171.54**
	90	$7.81±0.02^{aC}$	$8.37±0.02^{bC}$	$8.36±0.01^{bD}$	$8.39±0.01^{bcC}$	$8.37±0.01^{bB}$	$8.42±0.02^{cC}$	$8.39±0.01^{bcBC}$	$8.46±0.01^{dC}$	212.83**
	120	$7.75±0.01^{aB}$	$8.34±0.01^{bcC}$	$8.32±0.02^{bC}$	$8.37±0.03^{cdC}$	$8.35±0.01^{bcB}$	$8.40±0.01^{dC}$	$8.37±0.01^{cdBC}$	$8.43±0.02^{deC}$	216.65**
F value		12.57**	269.60**	575.19**	406.78**	329.58**	435.17**	456.74**	500.97**	

C - Control cheddar cheese with *Bifidobacterium bifidum (BB 12)*
T1 - 2% pectin incorporated cheddar cheese with *Bifidobacterium bifidum (BB 12)*
T2 - 1.5% inulin incorporated cheddar cheese with *Bifidobacterium bifidum (BB 12)*
T3 - 2.5% maltodextrin incorporated cheddar cheese with *Bifidobacterium bifidum (BB 12)*
T4 – 2% Pectin + 1.5% Inulin with *Bifidobacterium bifidum (BB 12)*
T5 - 2% Pectin + 2.5% Maltodextrin with *Bifidobacterium bifidum (BB 12)*
T6 - 1.5% Inulin + 2.5% Maltodextrin with *Bifidobacterium bifidum (BB 12)*
T7 - 2% Pectin+ 1.5% Inulin+ 2.5% Maltodextrin with *Bifidobacterium bifidum (BB 12)*
Means bearing different superscripts within rows (a, b, c, d) differ significantly ($P< 0.05$) (NS- $P > 0.05$; *- $P< 0.05$; **- $P< 0.01$)
Means bearing different superscripts within columns (A, B, C, D) differ significantly ($P< 0.05$) (NS- $P > 0.05$; *- $P< 0.05$; **- $P< 0.01$)

Conclusion

The prebiotics *viz.* pectin, inulin and maltodextrin when incorporated at the recommended levels, namely 2.0%, 1.5% and 2.5% respectively helped in enhancing the viable count of both *Lactobacillus acidophilus* (LA 5) and *Bifidobacterium bifidum* (BB 12). The maximum total viable count was attained during 60 days of storage for both *Lactobacillus acidophilus* (LA 5) and *Bifidobacterium bifidum* (BB 12). Post 60 days of storage, though the microbial count decreased gradually, the level did not fall below 10^7 cfu / g throughout the whole period of storage.

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