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Viability of *Lactobacillus Acidophilus (LA 5)* in synbiotic cheddar cheese slice incorporated with prebiotics during storage

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Abstract

Cheddar cheese is a hard type of cheese and source of protein, fat, calories and vitamins to consumer. Cheese slice with optimum level of prebiotics is known to support the growth of probiotic microorganisms. To evaluate the effect of prebiotics on the growth and viability of *Lactobacillus acidophilus (LA 5)* different treatments with selected levels of pectin (0.3%), inulin (0.2%) and maltodextrin (0.4 %) were analyzed at two different packaging conditions. The results indicated that the treatments with 0.3% Pectin+ 0.2% Inulin+ 0.4 % Maltodextrin incorporated cheddar cheese slice showed higher viable counts. The results indicated that at the end of 120 days storage period, *L. acidophilus (LA 5)* count (Log_{10}cfu/g) was 8.48±0.02 and 8.43±0.02 in aerobic and vacuum package, respectively. The results confirmed that the supportive action of pectin, inulin and maltodextrin as growth promoter for *L. actobacillus (LA 5)* in cheddar cheese slice.

Keywords: Cheddar cheese, prebiotics, viability, *Lactobacillus acidophilus (LA5)*, aerobic and vacuum package

Introduction

Dairy products provide a package of essential nutrients that are required by our body and hence dairy foods have been an important part of the human diet. Due to its physico-chemical characteristics, cheese proved to possess several advantages as a carrier for probiotics when compared to other fermented milk products. Processed cheese tops the table in total supermarket cheese sales, followed by Cheddar and Mozzarella type of cheeses (Kapoor & Metzger, 2008) [3]. The majority of the probiotic bacteria in use today belong to the genera *Lactobacillus* and *Bifidobacterium* but products containing strains from other genera such as *Propionibacterium*, *Enterococcus* and *Escherichia* are also available (Marco *et al.*, 2006, Minocha, 2009) [8]. Synbiotic or probiotic dairy products are considered to be healthier and its increased demand shows that the consumer's inclination towards functional dairy products for diversified health benefits apart from nutritional value has paved the way to develop synbiotic cheese slice incorporated with probiotics *viz*. *Lactobacillus acidophilus* and prebiotics such as pectin, Inulin and maltodextrin. Extensive research on the important physicochemical and functional properties has resulted that probiotic cheese is one of the most versatile dairy products with numerous end-use applications.

Inulin and fructo-oligosaccharides are more important ingredients of prebiotics contained in foods (Zamora *et al.*,2013) [11]. Yasmin *et al.* (2018) [9] tried whey protein concentrate-pectin-alginate based delivery system to improve survival of *B. longum* BL-05, the study revealed that extrusion using whey protein concentrate and pectin as encapsulating material could be considered as one of the novel technologies for protection and effective delivery of probiotics. Mali *et al.* (2020) [6] studied the nutritional qualities of synbiotic cottage cheese using *Lactobacillus acidophilus (LA-5)* and *Lactobacillus casei (NCDC-298)* with pectin concluded that developed synbiotic cottage cheese had better nutritional qualities than that of the control.

Bisar *et al.* (2015) [1] produced a synbiotic fermented dairy product with the polysaccharides fibres (maltodextrin, polydextrose and inulin) with a 3% concentration in combining them with *Bifidobacterium longum ATCC 15707*, *Streptococcus thermophilus Texl 5842* and *Lactococcus lactis subsp lactis ATCC 53214* in either skimmed or full fat fermented milk. Hence, the research was conducted to develop synbiotic cheddar cheese slice using different prebiotics i.e pectin, inulin and maltodextrin and to evaluate the viability of *Lactobacillus acidophilus (LA 5)* incorporated with inulin, pectin and maltodextrin during storage.

Materials and Methods

1. Manufacturing of cheddar cheese slice

Cheese was prepared according to the standard procedures mentioned in Khalaileh and Ajo (2013) [4] with minor modifications. After standardization of milk, the prebiotics (selected levels of pectin (0.3%), inulin (0.2%) and maltodextrin (0.4 %) (fig.1) were added and the pasteurized at 63°C for 30 min and then cooled to 30°C. A cheese starter culture consisting of *Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris* was used as starter culture for acid production during cheddar cheese making. The pre-activation of probiotic was performed as per the methodology described by Buriti *et al.* (2007) [2] with adaptations. It was done as follows: 0.02g each of LA – 5 DVS probiotic cultures were added to 40 mL of reconstituted skim milk separately. Then the cultures were incubated at 37°C for 2.5 hours under anaerobic condition. The probiotic is added at the rate of 5% for preparation of cheddar cheese. The cheddar cheese slices were packed under aerobic and vacuum conditions. The cheddar cheese was stored at 4±1°C for varying periods and analyzed on 0^{th}, 30^{th}, 60^{th}, 90^{th} and 120^{th} days.

Pectin **Inulin** **Maltodextrin**

Fig 1: Prebiotics (Pectin, Inulin and Maltodextrin)

2.2 Enumeration of *Lactobacillus acidophilus (LA 5)* in cheddar cheese slice

The cheese slice samples (10g) were diluted in 90 mL of sterile distilled water. The samples were macerated in mortar and pestle to obtain slurry for the first dilution and subsequent serial dilution were done. The media MRS agar with Bromocresol green and clindamycin was used for *Lactobacillus acidophilus (LA5)* Bromocresol green stock solution was prepared at 0.2% (w/v), autoclaved at 121ºC for 15 min and added at the rate of 20ml/L to the autoclaved molten agar base. Clindamycin stock solution, prepared by dissolving 5mg in 100ml distilled water and sterilized and added at the rate of 2ml/L to the autoclaved base.

Experimental design for the study is as follows

Treatments	Description
Control	Cheddar cheese with *Lactobacillus acidophilus* (LA 5)
T_1	0.3% Pectin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)
T_2	0.2% Inulin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)
T_3	0.4% Maltodextrin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)
T_4	0.3% Pectin + 0.2% Inulin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)
T_5	0.3% Pectin + 0.4% Maltodextrin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)
T_6	0.2% Inulin + 0.4% Maltodextrin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)
T_7	0.3% Pectin+ 0.2% Inulin+ 0.4 % Maltodextrin incorporated cheddar cheese slice with *Lactobacillus acidophilus* (LA 5)

2.3 Sub-culturing of *Lactobacillus acidophilus (LA 5)* in skimmed milk medium

Sub-culturing was done in the medium containing 11 % skimmed milk powder which was reconstituted with demineralized water and autoclaved at 121° Cfor 15 minutes. The medium was checked for sterility for 24 hours and then inoculated with mother culture and incubated at 37°C aerobically until the formation of coagulum. After formation of coagulum, culture was stored at 4°C for periodical transfer.

The cheddar cheese slices were packed under aerobic and vacuum condition (Plate 2). The cheddar cheese was stored at 4±1°C for varying periods and analyzed on 0^{th}, 30^{th}, 60^{th}, 90^{th} and 120^{th} days.

Results and Discussion

2.4.1 Viable Count of *Lactobacillus acidophilus (LA 5)* in aerobically and vacuum packed synbiotic cheddar cheese slice incorporated with prebiotics during storage

The results of this study revealed from Table 1. that a highly significant (P≤0.01) increase in viable count of L. *acidophilus* from day 0 to 60 days of storage for all the treatments and the control showed significant difference between storage periods. The highest count was observed for T_7 (8.78 ± 0.02) on 60^{th} day of storage and the count

Table 1: Mean (± SE) Viable Count of *Lactobacillus acidophilus (LA 5)* in aerobically packed cheddar cheese slice incorporated with prebiotics during storage

Probiotic	Days	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	F value
L. acidophilus count (Log $_{10}$cfu/g)	0	7.71^{abE} ±0.06	7.99^{bcD} ±0.11	7.55^{cF} ±0.02	8.27^{aB} ±0.01	8.13^{bC} ±0.01	8.52^{cA} ±0.01	8.45^{aA} ±0.01	8.53^{dA} ±0.01	180.55 **
	30	7.79^{aH} ±0.01	8.02^{abF} ±0.01	7.86^{aG} ±0.01	8.29^{aD} ±0.00	8.14^{bE} ±0.01	8.55^{bB} ±0.02	8.49^{bC} ±0.01	8.72^{bA} ±0.02	302.66 **
	60	7.83^{aH} ±0.02	8.03^{aF} ±0.01	7.88^{aG} ±0.01	8.31^{aD} ±0.01	8.18^{aE} ±0.01	8.63^{aB} ±0.03	8.54^{cC} ±0.01	8.78^{aA} ±0.02	479.60 **
	90	7.76^{aF} ±0.04	7.95^{cE} ±0.01	7.70^{bF} ±0.01	8.25^{aC} ±0.04	8.03^{cD} ±0.01	8.55^{bB} ±0.02	8.47^{dC} ±0.01	8.70^{cA} ±0.04	316.26 **
	120	7.62^{bF} ±0.06	7.88^{dE} ±0.02	7.49^{dG} ±0.02	8.17^{B} ±0.03	7.95^{dD} ±0.01	8.42^{dA} ±0.03	8.38^{eC} ±0.01	8.48^{eA} ±0.02	424.45 **
F value		4.166*	16.031**	109.67**	155.17**	60.72**	34.74**	224.69**	43.82**	

Means (n=6) bearing uppercase superscripts between columns differ significantly (P<0.05)

Means (n=6) bearing lowercase superscripts between rows differ significantly (P<0.05)

(P< 0.05) * = Significant difference; (P≤ 0.01) ** = Highly significant difference;

Table 2: Mean (± SE) Viable Count of *Lactobacillus acidophilus (LA 5) in* vacuum packed cheddar cheese slice incorporated with prebiotics during storage

Probiotic	Days	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	F value
L. acidophilus count (Log	0	7.70^{bF} ±0.04	7.97^{cE}±0.01b	7.52^{cG} ±0.01	8.24^{cC} ±0.01	8.10^{bD} ±0.02	8.50^{cA} ±0.01	8.42^{bB} ±0.04	8.51^{bA} ±0.02	418.70**
	30	7.79^{aH} ±0.01	7.99^{abF} ±0.01	7.83^{aG} ±0.01	8.27^{abDE} ±0.01	8.14^{a} ±0.01	8.55^{bB} ±0.02	8.43^{bC} ±0.02	8.72^{aA} ±0.01	1187.85**
	60	7.80^{aH} ±0.02	8.01^{aF} ±0.02	7.85^{aG} ±0.02	8.29^{aD} ±0.02	8.15^{aE} ±0.01	8.62^{aB} ±0.01	8.46^{aC} ±0.01	8.75^{aA} ±0.01	1084.38**

$_{10}$cfu/g)	90	7.76^{abG} ±0.01	7.95^{cF} ±0.03	7.70^{bH} ±0.01	8.25^{cD}±0.02b	8.03^{cE} ±0.01	8.55^{bA} ±0.02	8.30^{cC} ±0.01	8.50^{bB} ±0.01	817.46**
	120	7.62^{cG} ±0.02	7.88^{dF} ±0.02	7.49^{cH} ±0.01	8.17^{dC} ±0.02	7.95^{dEF} ±0.01	8.42^{dA} ±0.01	8.12^{dD} ±0.01	8.43^{bA} ±0.02	810.29**
F value		13.44**	24.21**	171.98**	25.49**	55.66**	35.36**	145.93**	142.07**	

Means (n=6) bearing uppercase superscripts between columns differ significantly (P<0.05)
Means (n=6) bearing lowercase superscripts between rows differ significantly (P<0.05)
(P< 0.05) * = Significant difference; (P≤ 0.01) ** = Highly significant difference;

significantly decreased to 8.48 ± 0.02 on 120th day of storage. The lowest count was observed for control (7.62± 0.06) on 120th day. A significant difference (P<0.05) was noticed between 0, 30, 60, 90 and 120 days of storage periods and between different treatments. Due to action of pectin, inulin and maltodextrin as growth promoter for *L. acidophilus* count in Cheddar cheese slice was higher. The results concurred with the findings of Yeo and Liong (2010) [10]. Pectin was utilized as a carbon source by the specific strains of yoghurt which resulted in proportional rise in *L. acidophilus* count (Lee *et al.*, 2013) [5].

It was observed from Table No.2 there was significant difference (P<0.05) in viable count of *L. acidophilus* during different days of storage. Similarly, significant difference (P<0.05) was also noticed among different treatments. There was highly significant (P≤ 0.01) increase in viable count from day 0 to 60 days of storage for all the treatments was observed. The highest count was noticed for T_7 (8.75 ± 0.01) on 60th day of storage and the viable count significantly decreased to 8.43 ± 0.02 on 120th day of storage. The lowest viable count was observed for control cheddar cheese slice (7.62 ± 0.02) on 120th day. Yeo and Liong (2010) [10] demonstrated that the growth of *L. acidophilus* could be stimulated in the presence of FOS and this could be due to the ability of secretion of the enzyme ß-fructofuranosidase enzyme by the probiotic.

Conclusion

The above results showed that viability count was higher in the treatments than the control and confirmed the supportive action of pectin, inulin and maltodextrin as growth promoter for *L. acidophilus (LA 5)* in Cheddar cheese slice.

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