Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 9, No. 1, 77-84, 2019

The Vaibility of Probiotic Bacteria and Characteristics of Un-cultured Cream Containing Inulin

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Received: 1 October 2017 Accepted: 18 December 2017

ABSTRACT: The effect of different levels of inulin (0, 1.5 and 3 %) and probiotic bacteria including *Lactobacillus acidophilus* and *Lactobacillus casei* on chemical properties (pH and titratable acidity) and viable count of probiotic strain of sweet cream during 30 days for 15-day interval at 4°C were studied. All experiments were carried out in three replications. The results showed that during the refrigeration storage of samples, pH decreased and acidity increased significantly. The addition of inulin caused significant changes in pH, acidity and the viability of probiotic strains. By increasing the level of inulin to 3%, the pH of the cream samples decreased, and the acidity and counts of the probiotics increased significantly and this was more significant in *Lactobacillus acidophilus*, although the treatment containing *Lactobacillus acidophilus* had the highest acidity. The probiotic survival evaluation also indicated that inulin treatment at 3% level was the best treatment for increasing the viability of the probiotics in cream. The results showed that the addition of prebiotics, such as inulin, could play an important role in increasing the viability of probiotics. In general, changes in the chemical properties of sweet cream during the refrigeration were standard, which could be considered as a synbiotic product and sweet cream as a dairy product can also be considered as a good carrier for probiotic bacteria.

Keywords: *Inulin, Lactobacillus acidophilus, Lactobacillus casei, Synbiotic, Sweet Cream*.

Introduction

The increased awareness of the consumers about public health and their concern regarding the role of food in health, the consumption of functional foods has been increased (Alhart, 2003). One of the most promising aspects for the development of functional foods is the use of probiotics and prebiotics. Dairy products are one of the most important food carriers for probiotics, because milk, due to its specific characteristics, is a suitable matrix for useful microorganisms, as well as functional components such as prebiotics (Akin *et al*., 2007).

The most important health benefits of probiotics include immune system stimulation and enhancement (Cross, 2002), decreased serum cholesterol levels (Yeganezad *et al*., 2007), improved lactose digestion (Founden *et al*., 2000), improved calcium absorption, vitamin synthesis (Heenan *et al*., 2004), prevention of various cancers, especially colon cancer (Siro *et al*., 2008), prevention of growth and activity of pathogenic microbes (Pochapin, 2000;

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Tamime, 2005); increased nutritional value (Majeed & Prakash, 2007) and the synthesis of bacteriosins (Holzapfel & Schillinger, 2002).

The most common probiotic bacteria belong to the genus *Lactobacillus* and *Bifidobacterium*. *Lactobacillus casei* is a gram-positive bacterium, free of spores (Blandino *et al*., 2003). It has antioxidant properties (Saide & Gilliland, 2005), high resistance to fermented milk products and antimicrobial activity (Maiocchi, 2001). *Lactobacillus acidophilus* is a species of gram positive bacterium, homofermentative, microaerophilic species, fermenting sugars into lactic acid, and grows readily at rather low pH values (below pH of 5.0) and has an optimum growth temperature of around 37°C. *Lactobacillus acidophilus* occurs naturally in the human and animal gastrointestinal tract and mouth. (Vijayakumar *et al*., 2008). To enhance the survival and viability of probiotics in food and improve the nutritional and health effects more often, a combinations of prebiotics and probiotics are used (Vincenzo et al., 2016). A prebiotic, is indigestible in the intestines and fermented and also optionally stimulate the growth or activity of one or numbers of intestinal bacteria often probiotics (Donkor *et al*., 2007). Inulin is one of the most important dietary fiber and prebiotic that is used in food systems. The combination of fructan of indigestible and fermentable that enhances the calcium absorption and thereby improve the bone mineral density (Bosscher *et al*., 2006), reduce cholesterol levels in blood serum (Lopez Molina *et al*., 2005), increasing the viability of probiotics and stimulate their growth and activity (Gibson, 2004). One of the dairy products that has the potential to preserve and transport probiotics is cream. Cream is part of the milk that is relatively rich in milk fat content and has been isolated by milk creaming and converted into fat-free emulsion in milk (Vincenzo *et al*., 2016). Naturally, cream as a dairy product can also be considered as a suitable carrier for probiotic bacteria, in which both non-fermented and fermentative species can be considered.

By considering the importance of the use of probiotics in dairy products and the positive role of prebiotics in stimulating the growth and activity of probiotic and aspects such as increasing the quality and acceptability of the product to the consumer and enhance the nutritional value of food and since there is little research about the survival of probiotics in cream or milk fat products, in this study we found out the effect of the activity of Probiotic bacteria (*Lactobacillus acidophilus and Lactobacillus casei*) and the presence and absence of inulin (as a growth stimulant) on some of the chemical properties and viability of probiotics in sweet cream.

Materials and Methods

UHT cream of a dairy factory was obtained and commercial single strain lyophilized culture of *Lb. acidophilu*s (La-5) and *Lb. caesi* (*L. casei* 431) were supplied from Chr. Hansen Horsholm (Denmark). Inulin was obtained from Sigma Aldrich (USA), Man Rogosa Sharpe (MRS) agar and Ringer Salt Pills were purchased from Merck Chemical Company (Germany).

- Preparation of Probiotic strains

The probiotic culture of *Lactobacillus casei* and *Lactobacillus acidophilus* were inoculated separately in 50 ml sterilized broth MRS medium (37° C) for 24 hours. At the end of this period, activated cells were isolated by centrifugation at 3000 G for 5 minutes at 25 °C and washed twice with sterile Ringer Salt solution with the same procedure. The cells were placed in a suitable volume of sterile distilled water, resulting in a concentration equivalent to 10^8CFU / ml.

- *Sample preparation*

Inulin was added at three concentrations (0, 1.5 and 3%) into sterile cream. Probiotic bacteria were inoculated separately into (1% v/v) cream, and Prepared samples were kept at 4 °C for 30 days for performance of microbiological and chemical analysis during storage period at 15 day intervals .

- *Physicochemical analysis*

Titratable acidity of the samples ($^{\circ}$D: degree of dornic) was measured by titrating of 10 ml of sample with 0.1 N NaOH using phenol phetalein as indicator (Akin *et al*., 2007). All pH measurements were made using a digital pH meter with combined glass electrode and temperature probe. The pH-meter was calibrated using standard buffer solutions at pH 4.0 and 7.0 (Ostil et al., 2005).

- *Microbiological analysis*

At each sampling interval, one bottle was aseptically withdrawn and after vigorous shaking, 1ml of its content dispensed into 9 milliliter of quarter strength Ringer's solution (Merck, Germany). Following this, appropriate dilutions were made and subsequently pour-plated in duplicate order was performed onto a selective media. *Lb. acidophilus* and *Lb.casei* were counted in MRS (De Man, Rogosa and Sharpe) agar incubated aerobically at 37°C for 24-48 hours. After incubation, bacterial colonies between 30 and 300 were counted and the results expressed as colony forming unit per milliliter (cfu/ml) of the sample. The data presented are the means of results obtained from duplicate plates of the samples analysed in cfu/ml. (Rodrigueze, 2000).

- *Statistics analysis*

Data were collected in a completely randomized design (CRD) and the means were compared using the Duncan test at 5% level. For statistical analysis, SPSS software version 22 was used for drawing graphs from Microsoft Excel (2010).

Results and Discussion

- *Results of pH assessment*

Table 1 shows the changes of pH values of the samples, from the preparation time up to the end of their refrigerated storage period (30 days).

The effects of storage time, inulin levels, and the kind of probiotic bacteria strain on the pH of samples were compeletly significant for treatments after 15 and 30 days ($P<0.05$). According to Table1, on the first day, there were no significant differences between the pH of control and other samples. after 15 days the treatments with control sample differed significantly ($P<0.05$) and the lowest pH was related to the treatment containing *Lb. acidophilus*. In general, the pH of the control sample did not differ significantly after 15 days. At the 30 th day, the lowest and highest levels of pH belonged to the treatment containing *L.b acidophilus* and control samples, which had significant differences ($P<0.05$). The results of the mean comparison indicated that by passing the storage days, the pH values, in both samples, containing probiotic strains showed significant differences between the two consecutive assessments. The lowest pH was related to *Lb.acidophilus* and 3% inulin. In other words, the presence of this bacterium with the highest level of inulin caused the most fall in pH, which might define that inulin has more influence on *Lb. acidophilus* viability. This conclusion is consistent with the results of Oliveira et al. (2009), which reported pH of yogurt samples decreased in the presence of inulin.

Figure 1 shows the comparison of the effect of the probiotic bacteria on the pH of the samples. The differences between treatments containing *Lb.acidophilus* and *Lb. casei* and control samples (without probiotics) were completely significant

(P<0.05). The highest and lowest levels of pH were related to the control sample (6.7 ± 0.004) and *Lb. acidophilus* (6.60 ± 0.12) (Figure 1).

Figure 1 shows that the comparison of the mean interaction influence of storage time and inulin levels on the pH of the samples. there was no significant difference between the treatments on the first day. After 15 days of the storage, the highest pH value was observed for control samples, which had a significant difference with the treatments (P>0.05). The difference between treatments with control sample was significant at 30th day (P>0.05) and the highest pH value was related to the control sample. One of the reasons was the stimulation of probiotics by inulin as a prebiotic compound and increasing the metabolic activity of these microorganisms at higher levels (3%), which increased the activity of these bacteria. A significant decrease in the pH value of the condensed milk with the permeat was reported by some researchers in the presence of two prebiotic compounds lactulose and inulin (Donkor, 2007).

Table 1. Comparison of the effect of inulin and probiotic bacteria during storage on pH value of the cream

Storage time(day)	Samples	Inulin (%)		
		0	1.5	3
1	control	6.77±0.005^{a}	6.77±0.005^{a}	6.77±0.005^{a}
	Lb.casei	6.77±0.005^{a}	6.77±0.005^{a}	6.77±0.005^{a}
	Lb.acidophilus	6.76±0.005^{a}	6.76±0.005^{a}	6.76±0.005^{a}
15	control	6.77±0.005^{a}	6.77±0.005^{a}	6.77±0.005^{a}
	Lb.casei	6.66±0.001^{b}	6.65±0.015^{c}	6.51±0.017^{f}
	Lb.acidophilus	6.61±0.005^{d}	6.56±0.005^{e}	6.48±0.012^{h}
30	control	6.77±0.005^{a}	6.77±0.005^{a}	6.77±0.005^{a}
	Lb.casei	6.51±0.001^{f}	6.49±0.001^{g}	6.48±0.005^{h}
	Lb.acidophilus	6.49±0.001^{g}	6.48±0.005^{h}	6.47±0.001^{i}

Means in the same column with different superscripts are different (p<0.05). (Mean ± SD)

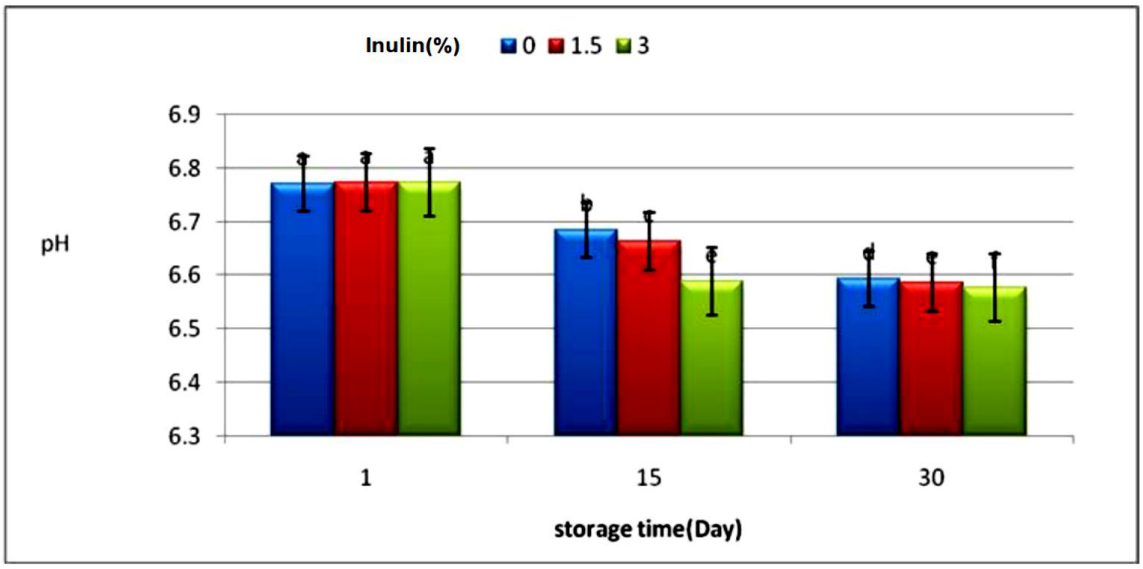

Fig. 1. Variation of the pH values in cream samples with different levels of inulin (0% control, 1.5% and 3.0% w/w) during 30-days of refrigeration

- *Titrable acidity*

The effect of storage time and inulin levels was quite significant on the titratiable acidity of the samples ($p<0.05$) (Table 2).

During cold storage (days 1, 15 and 30) and inulin levels (0, 1.5 and 3 percent), the acidity was increased. Figure 2 shows the Comparison of the effect of probiotic bacteria on the acidity of cream samples during 30 days refrigeration storage (Figure 2).

Interactions between storage time and inulin levels on the acidity of cream samples on the 15th and 30th day were statistically significant ($P<0.05$), but the treatments were not significantly different on the first day.

This result can be attributed to the more inulin-stimulating effect at higher levels and to the increased metabolic activity of probiotics in cream samples, especially *Lb.acidophilus*. In confirmation of the results of this study, Akin et al. (2007) reported that with increasing levels of inulin in ice cream, acidity significantly increased. Also, the results of studies by some researchers have shown a significant increase in the acidity of probiotic yogurt during the maintenance period (Vahcic & Hruskar, 2000). In general, the highest levels of acidity during the fifteenth and thirtieth days were for the highest level of inulin (3%) (Figure 3).

Table 2. Comparison of the effect of inulin and probiotic bacteria during storage on the titrable acidity of the cream

Storage time(day)	Samples	Inulin(%)		
		0	**1.5**	**3**
1	control	14.1±0.l	14.1±0.l	14.1±0.l
	Lb.casei	14.11±0.l	14.11±0.l	14.11±0.l
	Lb.acidophilus	14.11±0.l	14.11±0.l	14.11±0.l
15	control	14.1±0.l	14.1±0.l	14.1±0.l
	Lb.casei	15.43±0.058^{k}	16.1±0.1^{i}	16.73±0.2^{d}
	Lb.acidophilus	15.87±0.058 ^{j}	16.5±0.1^{f}	17±0.5^{b}
30	control	14.1±0.l	14.1±0.l	14.1±0.l
	Lb.casei	16.23±0.058^{h}	16.63±0.058^{e}	17±0. 1^{b}
	Lb.acidophilus	16.43±0.058^{g}	16.83±0.058^{c}	17.3±0.1^{a}

Means in the same column with different superscripts are different ($p<0.05$). (Mean ± SD)

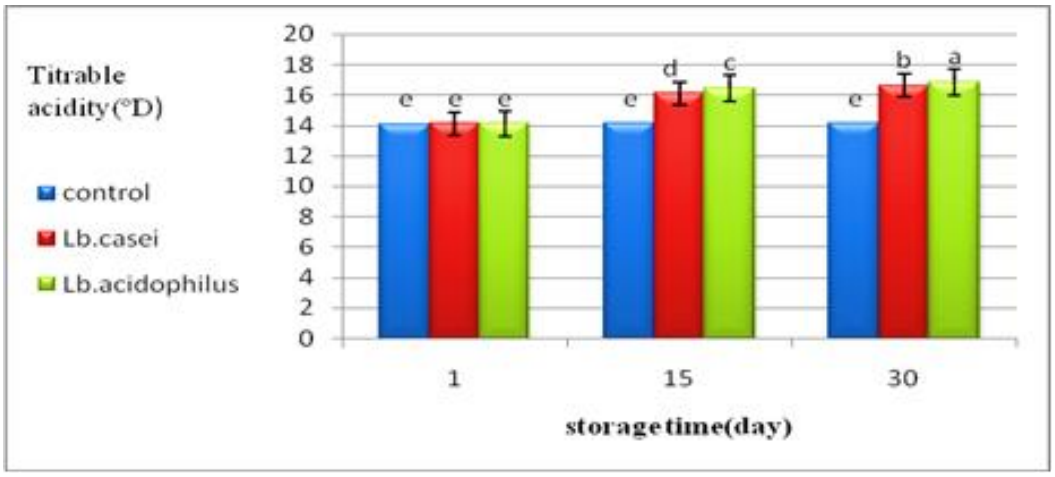

Fig. 2. Comparison of the effect of probiotic bacteria on the acidity of the cream samples during 30-days at refrigeration storage.

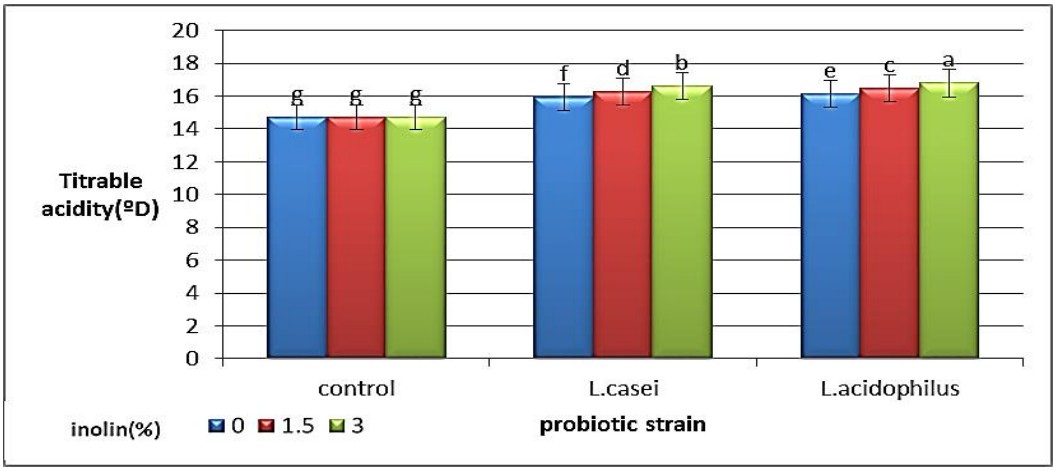

Fig. 3. Comparison of the effect of probiotic bacteria and inulin on the titrable acidity of the cream samples

The titrable acidity level in the probiotic and control samples during the storage did not differ significantly on the first day, while on the 15th and 30th days the difference in treatments was significant and the content of *Lb. casei* had the lowest acidity (Figure 4) The highest acidity in the final day was related to *Lb. acidophilus* content with 3% inulin, which had a significant difference with other treatments ($p<0.05$). on the first day, the effect of time, inulin, and probiotics on the level of acidity of all treatments was not significant, but on the fifteenth and thirtieth day, two treatments containing probiotics and different levels of inulin showed significant differences with the control sample ($P <0.05$).

According to Table3, during storage of cream samples from the first day until the end of the fifteenth day, the number of probiotic bacteria increased and after the fifteenth day, decreased significantly. The survival rate of *Lb. acidophilus* was significantly higher than that of *Lb.casei* and there were significant differences between the two treatments and the control sample on the 15th day ($P <0.05$). By adding probiotic bacteria to milk production, especially the fermentation, the growth rate and proliferation of these bacteria were reduced and their concentration decreased during cold storage.

By increasing of inulin levels (3%), the probiotic counts also increased, with the highest number of *Lb. acidophilus*, which had a significant difference with *Lb. casei* and control samples at all days ($P <0.05$). This result is clearly due to the effect of inulin on the role of the prebiotic compound that stimulates the growth, activity of probiotics used (Table 3).

Paseephol (2008) examined the bioavailability of *Lb. casei* LC-01 in yogurt in the presence of inulin for 28 days at a temperature of 4°C. The results showed that the inulin conjugate with *Lb.casei* increased the survival of this bacterium by more than 10^7 cfu / ml. Also, Donkor (2007) studied the survival time of *Lb.paracasei* L26 and *Lb.acidophilus* L10 and yogurt tissues in the presence of 0.5, 1 and 1.5% inulin for 28 days at a temperature of 4°C. The results showed an increase in viscosity and an increase in the number of probiotic bacteria (10^8CFU/ml).

According to Figure 4, by increasing the shelf life of cream samples until the 15th day, the number of probiotic bacteria increased and after the 15th day, a significant decrease was observed. The survival rate of Lb. acidophilus was significantly higher than that of Lb. casei and there was a significant difference between the two treatments and the control

Table 3. Surviving bacterial count (CFU/ml) in cream with different levels of inulin during 30-days cold storage

Storage time(day)	Samples	Inulin(%)		
		0	1.5	3
1	control	0 ± 0^{g}	0 ± 0^{g}	0 ± 0^{g}
	Lb.casei	$1.8\pm0\times10^{8\,f}$	$1.8\pm0\times10^{8\,f}$	$1.8\pm0\times10^{8\,f}$
	Lb.acidophilus	$1.87\pm0\times10^{8f}$	$1.87\pm0\times10^{8f}$	$1.87\pm0\times10^{8f}$
15	control	0 ± 0^{g}	0 ± 0^{g}	0 ± 0^{g}
	Lb.casei	$1.83\pm0.57\times10^{8\,e}$	$2.43\pm0.3\times10^{8\,ef}$	$6.87\pm0.2\times10^{8\,c}$
	Lb.acidophilus	$1.87\pm0.20\times10^{8f}$	$1.2\pm0.17\times10^{9b}$	$1.\ 73\pm0.5\times10^{9a}$
30	control	0 ± 0^{g}	0 ± 0^{g}	0 ± 0^{g}
	Lb.casei	$1.77\pm0.11\times10^{8\,f}$	$3.1\pm0.75\times10^{8\,e}$	$4.53\pm0.23\times10^{8\,d}$
	Lb.acidophilus	$2.27\pm0.37\times10^{8ef}$	$1.1\pm0.10\times10^{9b}$	$1.63\pm0.11\times10^{9a}$

Means in the same column with different superscripts are different ($p<0.05$). (Mean ± SD)

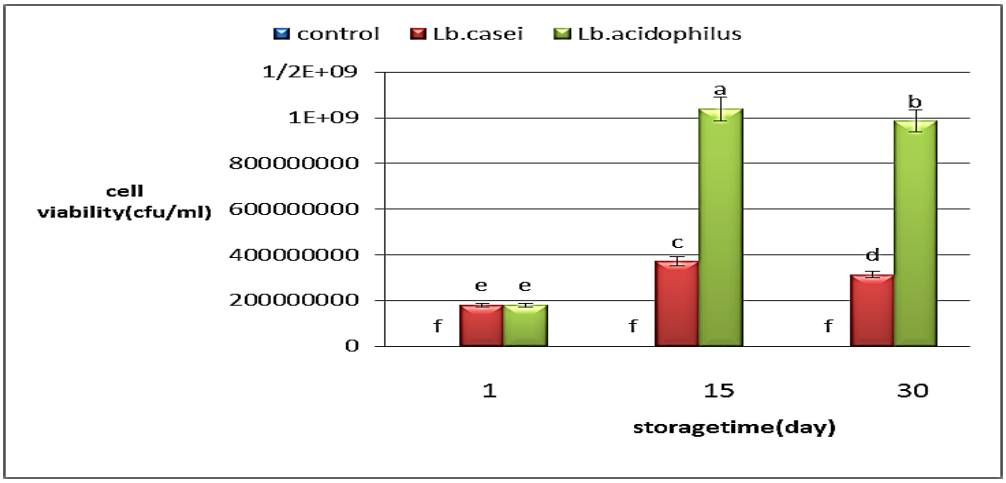

Fig. 4. Comparison of the effect of probiotic bacteria on the cell viability during cold storage

sample on the 15th day ($P<0.05$). By adding probiotic bacteria to milk production, especially the fermentation, after the fermentation stage, the growth rate of these bacteria are reduced and their amount decreased during cold storage.

Conclusion

In this study, inulin as prebiotic was added to the cream containing *Lactobacillus acidophilus* and *Lactobacillus casei*. The results showed that the application of higher levels (3%) of inulin increased the viability of these bacteria, especially *Lactobacillus acidophilus*.The acidity and pH, were increased and decreased respectively. Higher inulin levels stimulate more probiotics and, as the result increased the metabolic actidity. Therefore, acidity increased significantly in inulin containing treatments. In general, changes in the chemical properties of uncultured cream during the refrigeration storage were standard, which could be considered as a synbiotic product and sweet cream as a dairy product might be considered as a good carrier for probiotic bacteria.

Acknowledgement

This research was supported by Food Science Research Center, Islamic Azad University, Qazvin, Iran

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