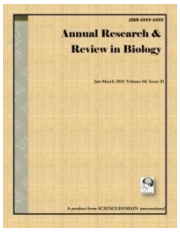

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Optimization of *Lactobacillus acidophilus* La-5, Feta Cheese Starters and Salt Content in Iranian Ultrafiltered Soft Cheese Formula

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAN performed all the experiments and wrote the first draft of the manuscript. Author MRE supervised the searches and provided the facilities for the use of pilot-plant UF system at membrane laboratory of Tehran University. Author AMS managed the literature searches and edited the manuscript analytically. Author KKD performed the statistical analysis, wrote the Taguchi design protocol and edited the manuscript statistically. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To optimize the process variables including: *Lactobacillus acidophilus* La-5, total starter (a mixture of mesophilic cheese starters (MCS) and thermophilic yoghurt starters (TYS), mesophilic cheese starter: thermophilic yoghurt starter (MCS: TYS) ratio and salt content in probiotic Iranian ultrafiltered soft cheese (IUSC).
Methodology: In order to choose the best probiotic cheese formula with the highest *Lactobacillus acidophilus* La-5 count, a total of eight samples with 20 % total solids were produced using a small scale ultrafiltration system. Probiotic bacteria of *Lactobacillus*

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acidophilus La-5 (0.1 and 0.3 %), total starters (1 and 2 %), MCS: TYS ratio (8:2 and 7:3) and salt (NaCl) concentration (1 and 2 %) by the help of Taguchi design were inoculated into cheeses and the viable cell counts of *Lactobacillus acidophilus* La-5 enumerated within 45 d of shelf life at 5 ºC, as well the multiple effects of process variables on probiotic count and colony morphologies of probiotic and starters evaluated.

Results: The most suitable conditions for the highest *Lactobacillus acidophilus* La-5 stability were addition of 0.1 % w/w *Lactobacillus acidophilus* La-5, 1 % v/w total starter, MCS: TYS ratio of 8:2 and 1 % w/w salt content. The lowest and highest viable cell count of probiotic *Lactobacillus acidophilus* La-5 in IUSC ranged from 6.41 to 7.92 $CFU.g^{-1}$, respectively. The acidified (pH 5.2) MRS agar medium used for the differentiation of the colony morphology of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* La-5, anaerobically. In all of the cheeses except for cheese 3 and 6 which the La-5 survived to 6.95 and 6.41 log orders, respectively, *Lactobacillus acidophilus* La-5 cell count survived to over10^7 $CFU.g^{-1}$, when all the cheeses had the small amount of acetic acid taste with a creamy and soft texture. The MCS:TYS ratio of 8:2 and 0.1 % initial dosage of *Lactobacillus acidophilus* La-5 ($P < 0.05$) affected the probiotic stability of *Lactobacillus acidophilus* La-5 in IUSC during shelf life, however the salt content (1 and 2 %) and total starter (1 and 2 %) did not significantly influence the *Lactobacillus acidophilus* La-5 viability.

Conclusion: This study showed that Iranian ultrafiltered soft cheese has a probiotic potential to have 10^7 $CFU.g^{-1}$ viable cell count of *Lactobacillus acidophilus* La-5 strain, when process variables optimized and this progress will contribute to develop a healthier foods.

Keywords: Probiotic; starter; soft cheese; ultrafiltration; medium; viability; La-5; Iran.

ABBREVIATIONS

Gastro Intestinal Tract = GIT, Iranian Ultrafiltered Soft Cheese = IUSC, Ultrafiltration = UF, Mesophilic Cheese Starters (Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris) = MCS, Thermophilic Yoghurt Starters (Streptococcus thermophiles and Lactobacillus bulgaricus) = TYS.

1. INTRODUCTION

The marked demand for 'healthy' foods is stimulating innovation and new product development in the food industry over whole the globe [1]. Functional foods provide healthy benefits to the host by achieving a balance in micro-flora of intestinal tract rather than traditional nutrient requirements, while probiotics are defined as live microbial supplements [2]. Probiotic bacteria, particularly bifidobacteria and lactobacilli are the normal inhabitants of the human colon. It is well studied that *Lactobacillus acidophilus* associated with several health and nutritional benefits in human beings.Their consumption has the potential to balance enteric flora [3], to reduce the cholesterol levels [4], to improve lactose digestion [5], to modulate immune systems [6], to provide anti-tumour effects [7], the production of vitamins [3], [8], [9], [10],and surface binding of toxins and heavy metals [11].

For provision of health benefits related to probiotic micro-flora, a suggested range for the minimum level for probiotic bacteria in probiotic cheese have been reported from 10^6 $CFU.g^{-1}$ [12] to 10^7 $CFU.g^{-1}$ [13]. Dairy products are now recognized for their nutritional benefits [14]. The production of functional foods have grown a rise of the interests concentrated on the incorporation of probiotic bacteria into cultured dairy products such as cheese and yogurt to

further improve the nutritional value of these products [15]. A wide range of probiotic dairy products has been studied including probiotic milk [16], yogurt [17], [18], cheese [19], [20], [21], and dough [22]. Cheese is a dairy product which has a considerable potential for delivery of probiotic organisms into the human GIT due to its important chemical and physical characteristics compared to fermented milks with higher pH value and lower titratable acidity, higher buffering capacity, fat content, nutrient availability and lower oxygen level, and also denser matrix of the texture [23], [24]. UF probably is one of the best alternative ways of concentrating milk before the formation and handling of the curd [25]. Application of UF for the production of cheese has been extensively studied [25], [26], [27], [28], [29], [30], [31], [32], [33], and also UF has been successfully applied in Feta cheese-making [28], [32]. IUSC made from bovine milk is manufactured from ultrafiltered and pasteurized milk with a mixed of mesophilic cheese, thermophilic yogurt starter cultures and commercial microbial rennet. Furthermore, evaluating the initial dosage of process variables to the ultrafiltered soft cheese including *Lactobacillus acidophilus* La-5, starters (total starter and mesophilic cheese and thermophilic yogurt starterratio as mixed starter cultures) and salt (NaCl) concentration may be useful to optimize the *Lactobacillus acidophilus* La-5 stability during shelf life. Yogurt starter culture is usually mixed with cheese starter in Iranian cheese-making plants, since the reduction in fermentation time desired. Turning to MCS:TYS ratio, Iranian dairy factories commercially use a mixture of mesophilic cheese and thermophilic yoghurt starter cultures with the proportion of 7:3, respectively. In order to lessen the amounts of thermophilic yoghurt starter as the main factor for cheese post-acidification and evaluate the different combinations of starter cultures on La-5 stability, the MCS:TYS ratio of 8:2 can be useful to be studied using Taguchi design. In addition, the proper amount of NaCl as an important element to enhance the taste of cheese or as a preservative agent should be taken into account, while it may decline the viable cell count of probiotic bacteria [34]. Since starter cultures grow competitively in cheese matrix, estimating the right amount of total starters and probiotic bacteria in cheese to achieve the highest stability of probiotic bacteria has not been studied [33].

Experimental design such as fractional procedure of Taguchi can be used as an efficient designing method in order toprovide reduced and reliable experiments based on the optimized responds [35], as it statistically determines the effects of multiple variables on a response. Several methods of experimental design can be used based on the number of main effects and interactions as well as their levels including: Full factorial, Plackett-Burman, and Taguchi design [36], [37], [38], [39], [40], [41], [42], [43]. The target of this work was to optimize cheese formula for the highest *Lactobacillus acidophilus* La-5 viability in ulterafilterd soft cheese using a pilot-plant scale of ultrafiltration system, to study the colony morphology of *Lactobacillus acidophilus* La-5 in the presence of starter cultures for the accurate enumeration and to evaluate the multiple effects of process variables including *Lactobacillus acidophilus* La-5, total starter, MCS:TYS ratio and salt content on the counts of *Lactobacillus acidophilus* La-5 during ripening. These results would be applicable to optimize the *Lactobacillus acidophilus* La-5 in development of probiotic-containing in IUSC. The main attributes of IUSC are the minimum of 20 % (w/w) total solids, a protein content of 6 %, fat content of 6 %, and a pH of 4.1- 4.7. This study is focused on the optimization of *Lactobacillus acidophilus* La-5 and Feta cheese starter cultures and accurate enumeration of probiotic bacteria in the presence of a mixed starters in IUSC. Optimized Iranian ultrafiltered-soft cheese showed that it can be used as a carrier of probiotic bacteria in order to benefit from some haelthy advantages which associated with *Lactobacillus acidophilus* La-5. The practical use of UF to manufacture the probioticsoft cheese in Iran has not been investigated yet.

2. MATERIALS AND METHODS

2.1 Cultures

Freeze-dried probiotic bacteria of *Lactobacillus acidophilus* La-5, R707 mesophilic cheese starters consisting of *Lactococcus lactis* ssp. *Lactis* and *Lactococcus lactis* ssp. *cremoris*, CH1 thermophilic yoghurt starters consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were obtained from Chr. Hansen (Horsholm, Denmark). Starters were rehydrated in sterile skim milkat 45 ºC for CH1 and 32 ºC for R707 starter before inoculation, while *Lactobacillus acidophilus* La-5 directly was added into retentate.

2.2 Cheese Making

Lots of 100 L milk with 2.5 percent fat and total microbial count less than 10^5 CFU.g^{-1} were pasteurized and cooled to 50 ºC for the UFprocess. Milk with concentration factor of 2 was then gradually ultrafiltered using a batch UF pilot system (Haddad Machine, Iran) equipped with an organic spiral-wound membrane (Polyethersulphone, Mimberfiltration, Germany). The attributes of the UF membrane werethe molecular weight cut-off of 20 kDa, 5.9 m^2 surface area, 1-2 bar equilibrium pressure in the singular module and 20 Litre total volume of tubes and module. The retentate was pasteurizedat 78 ºC for 1 min and homogenized at 65 ºC and 50 bar pressure. After the retentate cooled downto 34 ºC,the experimental cheeses were formulated by a combination of process variables (Table1), where in order to calculate the total starter, firstly the proportion of mesophilic cheese and thermophilic yogurt starters have calculated for each cheese formula (8:2 and 7:3) and then according to the Table 1 the dosage of prepared starters either 1 or 2 % v/w were formulated in cheeses. Thereafter, rennet was added into retentate and gently mixed with a sterile glass-bar near the flame. Curds were formed in cheese-cups within 15-20 min and they were then covered by parchment papers, salted withgranular salt and all cheese cupswere immediately packaged, sealed, turned and delivered to incubator at 27 ºC for 14-16 h. Finally, the cheeses were ripened andstored at 5 ºC for 45 d and evaluated microbiologically and chemically within refrigeration period.

Table 1. Cheese formulas and designed process variables using Taguchi design (N = 8)

Cheese formulas	Process variables			
	La-5* w/w %	Total starter** v/w %	(MCS***:TYS****) ratio	Salt w/w %
1	0.1	1	8:2	1
2	0.1	1	7:3	2
3	0.1	2	8:2	2
4	0.1	2	7:3	1
5	0.3	1	8:2	2
6	0.3	1	7:3	1
7	0.3	2	8:2	1
8	0.3	2	7:3	2

La-5: Lactobacillus acidophilus La-5; **Total Starter:(a mixture of mesophilic cheese and thermophilic yogurt starters); ***MCS: Mesophilic Cheese Starters(Lactococcus lactis ssp. lactis and Lactococcus lactis ssp.cremoris),'**TYS: Thermophilic Yogurt Starters (Streptococcus thermophiles and Lactobacillus bulgaricus)*

2.3 Cheese Formulations

The L8 program of Taguchi design was applied to formulate the eight ultrafiltered soft cheeses with four process variables including the dosage of probiotic bacteria, total starters, mesophilic cheese starter: thermophilic yogurt starter (MCS:TYS) ratio and salt (NaCl) concentration in two levels [35], as it shows in Table 1. Each row in the Table 1 belongs to one cheese formula which is automatically designed according to Taguchi design, where the dosage of process variables are different and all eight cheese formulas were fractionally designed.

2.4 Selection of Culture Media

In order to test the differential or selective media to enumerate the probiotic bacteria in the presence of starter cultures, different culture media were tested. *Lactobacillus acidophilus* La-5 and *Lactobacillus bulgaricus* were examined on acidified (pH 5.2) MRS agar [21], [44], [45], [46], *Streptococcus thermophilus* [47], *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* counts were performed on M17 agar [48,49]. Thereafter, the typical colony morphologies of probiotic bacteria and starters were then recorded.

2.5 Microbiological and Physico-Chemical Analysis

To enumerate the *Lactobacillus acidophilus* La-5 in experimental cheeses after 3, 7, 15, 30 and 45 d of cheese ripening, 20 g cheese of each cheese was transferred into a sterile bag under aseptic condition and homogenized in 180 mL of sterile trisodium citrate solution 2 % w/v; for 2 min using a Lab-blender 400 stomacher (Behin azma, Iran). Serial dilutions were prepared by adding 1 mL to 9 mL 0.1 % sterile peptone water (Merck, Germany) and plated following the pour-plate technique on different media. Samples were tested for the enumeration of starters and probiotic bacteria. *Lactococci* starters and *Streptococcus thermophilus* were aerobically enumerated on M17 agar at 25 ºC and 45 ºC for 3 d, respectively. *Lactobacillus acidophilus* La-5 and *Lactobacillus bulgaricus* were counted on acidified (pH 5.2) MRS agar (pH 5.2 was adjusted with HCl 0.1 N) anaerobically for 3 d at 45 ºC (Gas Pak system, Merck). Finally, coliform bacteria, yeast and mold were controlled during cheese ripening, and physico-chemical analysis of cheeses were done at 45 d of cheese ripening with the exception of pH value which controlled within time intervals (data not shown).

2.6 Sensory Evaluations

An informal sensory analysis of the cheeses was done using a non-trained taste-panel in the laboratory of the dairy factory and it aimed to score the eight samples and describe the differences and unfavorable characteristics of cheeses. The panelists answered the questionnaires with the ideas of good, average and unsuitable. Data collected and results described as overall points [44], (data not shown).

2.7 Statistical Analysis

To analyze the data from viable counts and pH value, one-way ANOVA procedure of SPSS statistical software (package version 19) was used [50]. The differences among means were detected by Duncan's multiple range tests.

3. RESULTS

3.1 Culture Media and Bacterial Colony Morphology

Fig. 1 shows the morphology characteristics of *Lactobacillus acidophilus* La-5 and lactic starters. The colony morphology of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* La-5 on acidified (pH 5.2) MRS agar were observed to be different to differentiate from each other. The cell recovery of *lactococci* and *Streptococcus thermophilus* starters on M17 agar illustrated the small, bright and creamy colour colonies 1-2 mm diameter and creamy colour and oval colonies but bigger size 1-4 mm diameter, respectively. On acidified (pH 5.2) MRS agar, *Lactobacillus acidophilus* La-5 yielded irregular clusters with beige creamy colonies, while colonies of *Lactobacillus bulgaricus* were round, smaller, disk form and white.

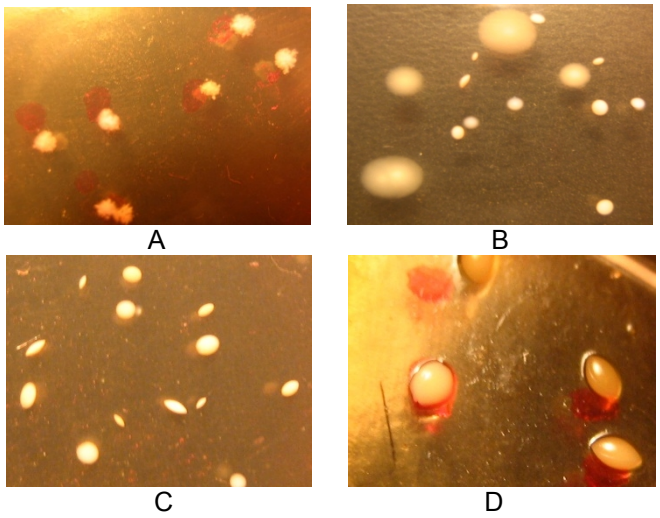

Fig. 1. The colony morphology of *Lactobacillus acidophilus* La-5 and starters in probiotic ulterafiltered soft cheese by different culture media and conditions

A: Lactobacillus acidophilus La-5 colony on MRS agar 5.2 at 45 ºC,72 h, anaerobiosis. B: Lactobacillus bulgaricus colony on MRS agar 5.2 at 45 ºC ,72 h, anaerobiosis.C: Lactococci starter colony of Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris, on M17 agar at 25 ºC, 72 h, aerobiosis. D: Streptococcus thermophilus colony on M17 agar at 45 ºC, 72 h, aerobiosis.

3.2 Changes in *Lactobacillus acidophilus* La-5 Count in Cheese Samples during Shelf Life

Fig. 2 indicates that in all of the cheeses except for cheese 3 and 6, *Lactobacillus acidophilus* La-5 cell count survived to over 10^7 CFU.g^{-1}. *Lactobacillus acidophilus* La-5 viability in cheese 1 was statistically different ($P < 0.05$) compared to other samples, when it showed the lowest changes in the number of *Lactobacillus acidophilus* La-5 by a rise of 0.11 log orders between 3 d and 45 d at 5 ºC, whereas the biggest fall for cheese 6 was recorded by loss of 1.68 log orders. In all of the cheeses except for cheese 3 and 6 which the La-5 survived to 6.95 and 6.41 log orders, respectively, *Lactobacillus acidophilus* La-5 cell count survived to over 10^7 CFU.g^{-1}. The *Lactobacillus acidophilus* La-5 count showed an initial increase for all cheeses from 3 d to 7 d and then gradually declined 1-2 log orders to the end of shelf life. The viable cell counts of probiotic bacteria of *Lactobacillus acidophilus* La-5

were 7.92, 7.65, 6.95, 7.01, 7.21, 6.41, 7.65 and 7.17 log CFU.g^{-1} in probiotic ultrafiltered soft cheeses 1, 2, 3, 4, 5, 6, 7 and 8, respectively.

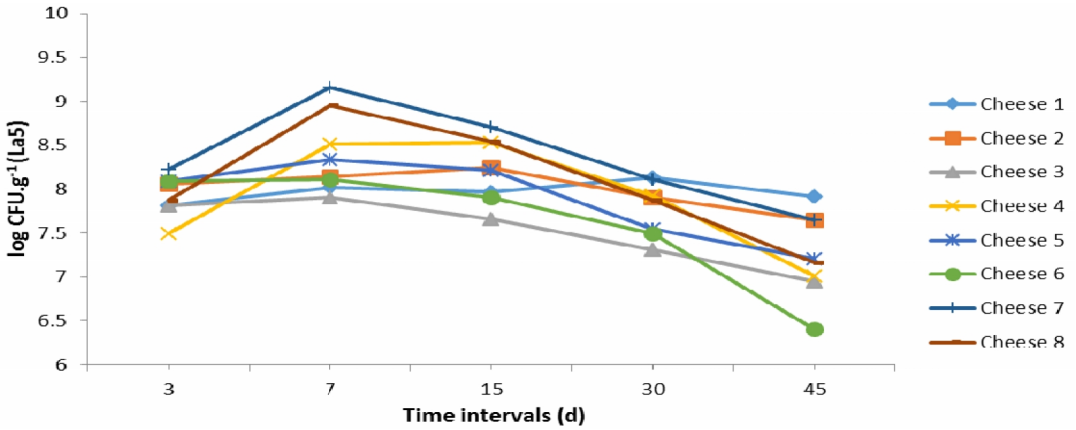

Fig. 2. The changes in the viability of *Lactobacillus acidophilus* La-5 (log CFU.g^{-1}) in eight ulterafiltered soft cheeses within time intervals (N=8)

3.3 pH Development of Cheeses and Physico-Chemical Parameters

Changes in pH for the probiotic cheeses recorded within the cheese ripening; it is noticeable that *Lactobacillus acidophilus* La-5 remained viable at pH ~ 4 in experimental cheeses to over 7 log orders within 45 d cheese ripening at 5 ºC. In all cases pH value stabilized to lower than 4.20 at 45 d. A significant (P < 0.05) increase in *Lactobacillus acidophilus* La-5 population was found for all samples in 7 d of cold storage, that went along with a significant decrease in pH values for all cheeses; however, the differences of pH among all cheeses were not significant at 45 d of cheese ripening (data not shown). The general values of physico-chemical parameters of probiotic ultrafiltered soft cheese evaluated as pH 4.12 ± 0.03, moisture 79.76 ± 0.74 % w/w, fat 5.11 ± 0.26 % w/w, protein 6.93 ± 06 % w/w, salt 2.11 ± 0.07 % w/w, ash 3.24 ± 0.08 % w/w and T.S 20.22 ± 1.12 % w/w.

3.4 Sensory Evaluations

Sensory evaluations of the studied cheeses showed that all probiotic cheeses were acceptable during shelf life, although they had asmall amount of acetic acid taste caused by *Lactobacillus acidophilus* La-5. In terms of the texture, all cheeses in this study showed a soft and creamy texture (data not shown).

3.5 Effects of Process Variables on *Lactobacillus acidophilus* La-5 Viability

In this study process variables including the *Lactobacillus acidophilus* La-5, total starter, MCS:TYS ratio and salt (NaCl) content showed different impacts on the *Lactobacillus acidophilus* La-5 viability at 45 d. These effects calculated positive (+) or negative (-) log orders of the enumeration of *Lactobacillus acidophilus* La-5.

Table 2 illustrates the multiple effects of two levels of designed process variables on the viable counts of *Lactobacillus acidophilus* La-5 at 45 d, according to the fact that bigger cell count is better.

Findings showed that the MCS:TYS ratio at level of 8:2, among the four process variables listed, has the most significant effect ($P < 0.05$) on the *Lactobacillus acidophilus* La-5 viability. The MCS:TYS ratio of 8:2 increased the La-5 count + 0.19 log orders from an average of 7.24 to 7.42 log orders, while the numbers decreased - 0.18 log orders with the proportion of 7:3. The higher *Lactobacillus acidophilus* La-5 stability may beso as to less existence of thermophilic yogurt starters in cheese that can cause higher post-acidification rate.

Table 2. The multiple effects of process variables (initial dosage of *Lactobacillus acidophilus* La-5, total starter, mesophilic cheese starter: thermophilic yogurt starter ratio and salt content) on the viability of *Lactobacillus acidophilus* La-5 (log CFU.g^{-1}) after 45 d at 5 ºC in probiotic ultrafiltered soft cheese formulas

Parameters	***Lactobacillus acidophilus* La-5 changes with 2 levels of variable (log CFU.g^{-1})**							
Variables	**La-5 %****		**Total starter %**		**MCS:TYS ratio*****		**Salt (NaCl) %**	
Levels	Level 1	Level 2	Level1	Level 2	Level 1	Level 2	Level 1	Level 2
	0.1 %	0.3 %	1 %	2 %	8:2	7:3	1%	2%
MaximumLa-5 viability	7.38	7.10	7.29	7.19	7.42	7.05	7.24	7.24
Average survivability of La-5	*7.24	*7.24	*7.24	*7.24	*7.24	*7.24	*7.24	*7.24
Changes of La-5 ***	+ 0.14	- 0.14	+0.05	-0.05	+0.18	-0.19	0	0

The means of survived Lactobacillus acidophilus La-5 influenced by different process variables compared to an average survivabilityof 7.24 (log CFU.g^{-1}) for all experimental cheeses. **La-5: Lactobacillus acidophilus La-5. * Changes of Lactobacillus acidophilus La-5. (+) changes enhance the La-5 stability, while (-) changes decline the La-5 viability according to the level of each process variable. ****MCS = Mesophilic Cheese Starters (Lactococcus lactis ssp. Lactis and Lactococcus lactis ssp. cremoris).TYS= Thermophilic Yogurt Starters (Streptococcus thermophiles and Lactobacillus bulgaricus)*

In contrast, NaCl concentration did not influence the La-5 viability in ultrafiltered soft cheeses during ripening, either with NaCl concentration of 1 or 2 % w/w and an average salt in dry matter (SDM) of 6 and 11 %, respectively. Initial dosage of *Lactobacillus acidophilus* La-5 of 0.1 and 0.3% w/w recommended by the provider company and cheeses formulated with these two levels. According to the results (0.1 % w/w) addition of *Lactobacillus acidophilus* La-5 showed a better performance, since counts of *Lactobacillus acidophilus* La-5 increased ($P < 0.05$)+ 0.14 log orders from an average count of 7.24 to 7.38 log orders, whereas with addition of 0.3% w/w of La-5, the viable cell count declined from an average of 7.24 to 7.10 log orders: a- 0.14 log orders fall, which might be due to the fact that lower population of probiotic bacteria can bring about the higher nutrient availability in the presence of mixed starters in the competitive cheese matrix. In terms of the effects of total starter dosage on *Lactobacillus acidophilus* La-5 viability, addition of 1 % v/w of total starter with no significant difference slightly increased the viable count of *Lactobacillus acidophilus* La-5, +0.05 log orders from an average of 7.24 to 7.29 log orders; however, 2 % v/w total starter caused negligible change in *Lactobacillus acidophilus* La-5 count (- 0.05 log orders).

4. DISCUSSION

In this work firstly, the colony morphology of probiotic bacteria of *Lactobacillus acidophilus* La-5 and cheese and yogurt cultures were tested in the different culture media and then the viability of *Lactobacillus acidophilus* La-5 were monitored in Iranian ultrafiltered soft cheese. Secondly,the dosage of *Lactobacillus acidophilus* La-5, starter cultures and salt content as process variables optimized for the production of cheese formula with the highest stability of *Lactobacillus acidophilus* La-5.

An important parameter for the accurate enumeration of microorganisms is the ability to count them either differentially or selectievely. Differential enumeration of probiotic bacteria

is difficult owing to the presence of several types of similar microbes in a product [51]. In this sense, we found that the acidified (pH 5.2) MRS agar medium was suitable to differentiate the colony morphology of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* La-5 anaerobically, as Dave et al. [45] previousely studied. Cheese starters of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* and yogurt starter of *Strptococcus thermophilus* did not grow in this medium, as Kasimoglu et al. [21] applied with probiotic white cheese. The cell recovery of *lactococci* and *Strptococcus thermophilus* starters on M17 agar were in agreement with the study of Lim et al. [49].

In order to achieve a reliable stability of probiotic bacteria, a convenient design of experiments such as Taguchi design could be taken into account. As long as many great dairy factories produce probiotic cheese each day, an optimazed formula can be highly cost-efficient for them. In this way, our finding indicated that the optimized formula of 0.1 % w/w *Lactobacillus acidophilus* La-5, 1 % v/w total starter, MCS:TYS ratio of 8:2 and 1 or 2 % w/w salt contenthad the highest stability of *Lactobacillus acidophilus* La-5 in IUSC to the end of 45 d cheese ripening.

The pH value as an indicator of cheese ripening evaluated during shelf life and there were no significant differences of pH values among all samples. It is noticible that *Lactobacillus acidophilus* La-5 remained stable at pH ~ 4 in the most of cheeses to over 7 log $CFU.g^{-1}$; although, as Blanchette et al. [52] reported previousely, with *Bifidobacteria,* the probiotic population rapidely dropped at pH = 4.5 of Cottage cheese during 28 d cheese ripening. This stability in viable count of La-5 at pH ~ 4 can be due to the rich source of nutrients for the growth of probiotic bacteria and starters such as small peptides and free amino acids caused by cheese proteolysis in parallel with proper activity water of ulterafiltered soft cheese. A signifiant increase in *Lactobacillus acidophilus* La-5 count was found at 7 d of cold storage, that went along with a significant decrease in pH values, as Bergamini et al. [44] previousely studied with semi-hard cheese.The acceptability is an important factor to sell probiotic products in the market, when compared to commercial non-probiotic products at supermarkets; in other words, their taste, aroma and texture should be similar to the non-probiotic products. According to Boylston et al. [23] and Gomes et al. [9], probiotic bacteria of *Lactobacillus acidophilus* and *Bifidobacteria* .spp produce acetic acid in dairy products. From our results,all the cheeses also were shown a small amount of acetic acid taste which was caused by *Lactobacillus acidophilus* La-5, but they were acceptable. Starter cultures and probiotic bacteria ferment lactose, remove oxygen and initiate the improved flavour in cheese and lead to decreased pH [53]. Turning to texture of the cheese, all cheeses had a soft and creamy texture.

Although a great variety of genera/ species and strain mixture of probiotic bacteria are exerted into different probiotic cheeses, multiple effects of process variables on the probiotic viability were barely reported. In this study, process variables including the *Lactobacillus acidophilus* La-5, total starter, MCS:TYS ratio and salt content showed different impacts on the *Lactobacillus acidophilus* La-5 viability at 45 d. The MCS:TYS ratio of 8:2 rather than 7:3, among the four process variables showed the most significant impacts ($P < 0.05$) on the *Lactobacillus acidophilus* La-5 viability, which is so as to the less amount of yogurt starters in cheese that can cause higher post-acidification rate.

NaCl concentration of IUSC generally is 2 % w/w in most of the dairy companies in Iran. So, because salt content can have an impact on the viability of probiotics, suggested 1 % w/w NaCl concentration for probiotic cheese by the cheese-making factories have applied to some of the cheeses so as to minimize the loss of *Lactobacillus acidophilus* La-5 during 45

days cold storage at 5 ºC. In contrast, salt dosage either 1 or 2 % showed the lowest effects on the *Lactobacillus acidophilus* La-5 viability in ultrafiltered soft cheeses. It was not observed any changes of *Lactobacillus acidophilus* La-5 count during ripening; however, salt content usually induces negative changes on the viability of probiotics in cheese [23]. According to previous results of Vinderola et al. [54], the NaCl concentration used 0.9 % in Argentinean probiotic cheese did not affect neither probiotic nor lactic acid starter bacteria viable cell count; however, usually NaCl content may induce negative changes on the viability of probiotics in cheese [23]. In addition, 0.1 % w/w addition of *Lactobacillus acidophilus* La-5 rather than 0.3 % w/w into IUSC showed a better performance, which may be due to the view that low population of probiotic bacteria can have higher availability of nutrients in order to remain stable with the mixed starters in cheese matrix.

In terms of the effects of total starter (a mixed of mesophilic cheese and thermophilic yogurt starters according to Table 1) on the *Lactobacillus acidophilus* La-5 viability, addition of 1 % v/w of total starter slightly increased the viable count of *Lactobacillus acidophilus* La-5, while 2 % v/w total starter caused negligible drop of *Lactobacillus acidophilus* La-5 count, as Vinderola et al. [33] investigated in the previous studies that *Lactobacillus delbrueckii* subsp*. bulgaricus* as starter weakely inhibited the growth of *Lactobacillus acidophilus* La-5 strain. In addition, the results of this study also showed that *Lactobacillus acidophilus* La-5 in IUSC were stable in the presence ofdifferent combinations of starter cultures. This work optimized the dosage of *Lactobacillus acidophilus* La-5, starter cultures and salt content and developed a new ultrafiltered soft cheese containing *Lactobacillus acidophillus* La-5 as a food grade vector of probiotic bacteria and allowed that to act as functional food, where *Lactobacillus acidophilus* La-5 in the most of samples survived to over 10^7 CFU.gr^{-1}.

The retentate with chemical analysis of 20 % T.S and 5 % fat and 6 % protein can also be used to study the influence of encapsulation of probiotic strains on probiotics physiology (such as digestive stress tolerance) and their morphology by comparing to the non-encapsulated probiotics in order to produce higher probiotic stability insoft cheese. In addition, this fermented soft cheese can be used in studies in which the prebiotics such as terhalose, yeast extract and inulin could enhance the expected health benefits, such as the immunomodulation in human beings.

5. CONCLUSION

This study optimized the formula of IUSC consisting of 0.1 % w/w *Lactobacillus acidophilus* La-5, 1 % v/w total starter, MCS:TYS ratio of 8:2 and either 1 or 2 % w/w salt content as process variables, when the highest cell count of *Lactobacillus acidophilus* La-5 is desired within 45 d of cheese ripening at 5 º C using Taguchi design. *Lactobacillus acidophilus* La-5 survived to over than 7 log orders in most of the samples and also all cheeses indicated a bit acetic acid taste caused by probiotic bacteria. In addition, all cheeses showed a soft and creamy texture. Finally, there is a potential to have high viable cell count of a *Lactobacillus acidophilus* La-5 strain in Iranian ultrafilterd soft cheese. Last but not least, successful progress of the ultrafiltered soft cheese containing *Lactobacillus acidophilus* La-5 will contribute to the evolution of healther foods with desirable sensory characteristics in modern food industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Beheshtipour H, Mortazavian AM, Haratian P, Khosravi-Darani K. Effects of *Chlorella vulgaris* and *Arthrospiraplatensis* addition on viability of probiotic bacteria in yogurt and its biochemical properties. Europ Food Research Technol. 2012;12:1798-1804.
2. Bergamini CV, Hynes ER, Quiberoni A, Suárez VB, Zalazar CA. Probiotic bacteria as adjunct starters: Influence of the addition methodology on their survival in a semi-hard cheese. Food Research Int. 2005;38:597-604.
3. Blancheette L, Roy D, Belanger G, Gauthier SF. Production of Cottage cheese using dressing fermented by *bifidobacteria.* J of Dairy Sci. 1996;79:8-15.
4. Boylston TD, Vinderola CG, Ghoddusi HB, Reinheimer JA. Incorporation of *Bifidobacteria* into cheeses: challenges and rewards, a review, Int Dairy J. 2004;14:375-387.
5. Cheryan M. Ultrafiltration and Microfiltration Handbook. CRC Press, Pennsylvania, Western Hemisphere.1998;231-260
6. Daigle A, Roy D, Belanger G, and Vuilmard JC. Production of probiotic cheese (Cheddar-like cheese) using enriched cream fermented by *Bifidobacterium infantis*, J of Dairy Sci. 1999;82:1081-1091.
7. Dave RI, shah NP. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus acidophilus* and *bifidobacteria.* J of Dairy Sci. 1996;79:1529-1536.
8. Deguchi Y, Morishita T, Mutai M. Comparative studies on synthesis of water soluble vitamins among human species of *Bifidobacteria*. Agricul and Biolo Chemi. 1985;49:13-19.
9. Donnet-Hughes A, Rochat F, Serrant P, Aeschilimann JM, Schiffrin EJ. Modulation of non-specific mechanisms of defense by lactic acid bacteria: Effective dose. J of Dairy Sci. 1999;82(5):863-869.
10. Esmaeili S, Khosravi-Darani K, Pourahmad R, Komeili R.An experimental design for production of selenium-enriched yeast, Worl Appl Sci J. 2012;19(1):31-37.
11. Farhadi S, Khosravi-Darani K, Mashayekh M, Mortazavian AM, Mohammadi A, Shahraz S. Production of propionic acid in a fermented dairy beverage, Int J of Dairy Technol. 2013;72:11-17.
12. Ferdousi R, Mohammadi R, Mortazavian AM, Khosravi_darani K, Homayouni-rad A. Evaluation of probiotic survivability in yogurt exposed to cold chain interruption. Iranian J of Pharmac Res. 2013;12 (Supplement):137-142.
13. Fox PF, McSweeney PLH, Cogen TM. Cheese, Chemistry, Physics and Microbiology. Third edition: General Aspects, chapman & Hall. 2004;1:234-290.
14. Fuller R. Probiotics 2, Applications and practical aspects, Chapman & Hall. 1997;135-204.
15. Gomes AMP, Malcata FX, Klaver FAM, Grande HG. Incorporation and survival of *Bifidobacterium* spp. strain Bo and *Lactobacillus acidophilus* Strain Ki in a cheese Product, Netherl Milk Dairy J. 1995;49:71-95.
16. Gomes AMP, Malcata. Development of probiotic cheese manufactured from goat milk: Response surface analysis via technological manipulation, J of Dairy Sci. 1998a;81:1462-1507.
17. Gomes AMP, Teixeira MG M, Malkata, F. X. Viablitiy of *Bifidobacteriumlactis* and *Lactobacillus acidophilus* in milk: Sodium chloride concentration and storage temperature. J of Food Processing and Preservation.1998b;22:221-240.
18. Gomes AMP, Malcata FX. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics, Trends in Food Sci & Technol. 1999;10:139-157.

19. Gomes AMP, Buriti FCA, Souza CHB, Faria JAF, Saad SMI. Probiotic Cheese: health benefits, technological and stability aspects. A review, Trends in Food Sci and Technol. 2009;20:344-354.
20. Green ML, Turvey A, Hobbs DG. Development of structure and texture in Cheddar cheese. J of Dairy Res. 1981;48:343-355.
21. International Dairy Federation. Detection and Enumeration of *Lactobacillus acidophilus*, IDF bulletin, No 306, Brussels, Belgium; 1995.
22. International Dairy Federation. Yogurt. Enumeration of characteristic micro-organism. Colony count technique at 37 ºC. Bulletin No. 117A/ 1988. IDF, Brussels, Belgium; 1988.
23. Ishibashi N, Shimamura S. *Bifidobacteria*: Research and development in Japan, Food Technol. 1993;47(6):126-136.
24. Jahadi M, Khosravi-Darani K, Ehsani MR, Mozafari MR, Saboury AA, Seyed ahmadian F, and Vafabakhsh Z. Evaluating the effects of process variables on protease-loaded nano-liposome production by Plackett-Burman design for utilizing in cheese ripening acceleration, Asian J of Chem. 2012;24(9):3891-3894.
25. Javanmard A, Rahmati-Roudsari M, Mortazavian AM, Sohrabvandi S, Khosravi-darani K. The impact of incorporation rate and order on physiological attributes of probiotic doogh, Int J of Pharmac Res. 2013;12(4):917-924.
26. Karami M, Ehsani MR, Mousavi SM, Rezaei, K, and Safari M. Changes in the rheological properties of Iranian UF-Feta cheese during ripening, Food Microbiol. 2009;112:539-544.
27. Kasimonglu A, Goncuoglu M, Akgun S. Probiotic white cheese with *Lactobacillus acidophilus*, Int Dairy J. 2014;14:1067-1073.
28. Khosravi-Darani K, Vasheghani-Farahani E, Shojaosadati A. Application of the Plackett-Burman Statistical Design to optimize poly (β-hydroxybutyrate) production by Ralstoniaeutropha in batch culture, Iranian Jof Biotech. 2003;1(3):155-161.
29. Khosravi-Darani K, Zoghi A. Comparison of pretreatment strategies of sugarcane bagasse: Experimental design for citric acid production, Bioresource Technol. 2008a;99:6986–6993.
30. Khosravi-Darani K, Zoghi, A, Alavi SA, Fatemi SSA. Application of Plackett Burman design for citric acid production from pretreated and untreated wheat straw, Iranian J of Chem and Chem Eng. 2008b;27(1):91-104.
31. Leievre J, Renner E. Manufacture of cheese from milk concentrated by ultrafiltration, J of Dairy Res. 1988;55:465-477.
32. Lim KS, Huh CS, Beak YJ. A selective enumeration medium for *bifidobacteria* in fermented dairy products. J of Dairy Sci. 1995;78:2108 -2112.
33. Lin M, Savaiano D, Harlander S. Influence of non-fermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. J of Dairy Sci. 1991;47(1):85-95.
34. Mattila-Sandholm T, Myllàrinen P, Crittenden R, Mogensen G, Fondėn R, Sarrella M. Technological challenges for future probiotic foods, Int Dairy J. 2002;12:173-182.
35. Mistry VV, Maubios JL. Application of membrane separation technology to cheese production. In P. F. Fox (Ed.). Cheese: Chemistry, physics and microbiology. London, UK: Chapman and Hall.1993;1:493-522.
36. Mohseni M, Ehsani MR, Mohammadi-Sani A. Survival of Bb12 and La5 in symbiotic milk, J of Nutr and Food Sci. 2013;43(2):137-141.
37. Mokhtari-Hosseini ZB, Vasheghani-Farahani E, Shojaosadati SA, Karimzadeh R, Khosravi-DaraniK. Media selection for poly (hydroxybutyrate) production from methanol by *Methylobacterium extorquens* DSMZ 1340, Iranian J of Chem and Chem Eng. 2009a;224-229.

38. Mokhtari-Hosseini ZB, Vasheghani-Farahani E, Heidarzadeh-Vazifekhoran A, Karimzadeh R, Khosravi-DaraniK.Statistical media optimization for growth and PHB production from methanol by a methylotrophic bacterium, Bioresource Technol. 2009b;28(3):45-52.
39. Nord, C. E., Lidbeck, A., Orrhage, K., and Sjöstedt, S. Oral supplementation with lactic-producing bacteria during intake of clindamycin. Clin microbiol and infection. 1997;3(1):24-132.
40. Qualitek-4 (ver: 0512). Automatic design and analysis of Taguchi experiments. Nutek, Inc. Bloomfield Hills, Michigan, USA; 2007. Available at http://www.nutek-us.com.
41. Rao DV, and Renner E. Studies on the application of ultrafiltration for the manufacture of Cheddar cheese. Effect of heating UF whole milk concentrate on composition and yield. Milchwissen.1988;43:708-711.
42. Reddy BS, Rivenson A. Inhibitory effect of *Bifidobacterium longum* on colon , mammary and liver carcinogenesis induced by 2-amino-3-methyl imidazo (4,5,F) quinoline, a food mutagen , Canc Res. 1993;53:3914-3918.
43. Renner E, Abd-El-Salam MH. Application of Ultrafiltration in the Dairy Industry, Elsevier Applied Science.1991;208-220.
44. Robinson RK. Microorganisms of fermented milks. In R. K. Robinson (Ed), Therapeutic properties of fermented milks,. London, UK: Elsevier Applied Science. 1991;23-43
45. Roy D. Media for the isolation and enumeration of *bifidobacteria* in dairy products. Int J of Food Microbiol. 2001;69:167-182.
46. Shah NP. Functional cultures and health benefits. Int Dairy J. 2007;17(11):1262-1277.
47. SPSS. Version 19 for windows. Chicago, Release 19; 2010. SPSS Inc.
48. Stanton CG, Gardiner PB, Lynch JK, Collins G, Fitzgerald P, Ross RP. Probiotic cheese. Int Dairy J. 1998;8:491-496.
49. Tamime AY. Microbiology of starter cultures, In R. K. Robinson (Ed), Dairy microbiology handbook (3rd Ed). New York, NY: Wiley. 2002;261-366)
50. Tamime AY, Robinson RK. Feta and related cheeses. England: Ellis Horwood Ltd. 1991; 70-143.
51. Tharamaraj N, Shah NP. Selective Enumeration of *Lactobacillus delbruckii* spp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacteria*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *propionibacteria*, J of Dairy Sci. 2003;86(7):2288-2296.
52. Vinderola CG, Mocchiutti P, Reinheimer JA. Interaction among lactic acid starter and probiotic bacteria used for fermented dairy products, J of Dairy Sci. 2002;85:721-729.
53. Vinderola CG, Prosello W, Ghiberto D, Reinheimer JA. Viability of probiotic (*Bifidobacterium, Lactobacillus acidophilus* and *Lactobacillus casei*) and non probiotic microflora in Argentinean fresco cheese.J of Dairy Sci. 2000;83:1905-1911.
54. Zoghi A, Khosravi-Darani K, Sohrabvandi S. Surface binding of toxins and heavy metals by probiotics. A mini-review in Medic Chem. 2014;14:84-98.

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