



## Scientific Research

## The effect of inulin and calcium chloride on the viscosity and color characteristics of synbiotic ricotta cheese

Marjaneh Sedaghati\*, Reyhaneh Kavand

1- Department of Food Science and Technology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran

2- MS student of Food Science and Technology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO	ABSTRACT
<p><b>Article History:</b></p> <p>Received: 2024/8/27</p> <p>Accepted: 2024/12/30</p> <p><b>Keywords:</b></p> <p>cheese, color indexes, inulin, ricotta, and viscosity.</p> <p><b>DOI:</b> 10.22034/FSCT.22.162.185.</p> <p>*Corresponding Author E- marjanehsedaghati@yahoo.com</p>	<p>This research was conducted with the aim of investigating changes in viscosity and color characteristics of synbiotic ricotta cheese with the addition of inulin and calcium chloride during the storage time. To produce synbiotic ricotta cheese, whey, <i>Lactobacillus acidophilus</i>, inulin, and calcium chloride were used. Inulin at three levels of 0, 1% and 3% and calcium chloride salt at three levels of 0, 150 and 300 mg/L were applied and changes in viscosity and color (color indexes L*, b* and a*) and the survival of <i>Lactobacillus acidophilus</i> during the storage investigated. The results showed the addition of inulin and calcium chloride significantly increased the viscosity of synbiotic ricotta cheese (<math>p &lt; 0.05\%</math>). The diagram of apparent viscosity-shearing rate indicated that with increasing shearing rate, the apparent viscosity of the samples decreased; Therefore, synbiotic ricotta cheese are non-Newtonian food. In the evaluation of the rheological parameters of different synbiotic ricotta cheese, it was found the cheese had a high correlation with the power law model and the synbiotic ricotta cheese showed pseudoplastic behavior. The changes of L* color index of synbiotic ricotta cheese revealed the addition of inulin to cheese significantly increased the L* color index and the presence of calcium chloride decreased the L* color index of synbiotic ricotta cheese significantly (<math>p &lt; 0.05</math>). Also, increasing the concentration of inulin and calcium chloride in different synbiotic ricotta cheese was associated with a significant decrease in b* and a* color index of cheese (<math>p &lt; 0.05</math>). According to the results, the addition of inulin and calcium chloride improved the survival of <i>Lactobacillus acidophilus</i> (<math>p &lt; 0.05</math>). According to the results, in the synbiotic ricotta cheese containing 3% inulin and 300 mg/L calcium chloride, the viscosity and color characteristics of the sample was favorable. In addition, this sample had the highest survival rate of <i>Lactobacillus acidophilus</i> among the synbiotic ricotta cheese.</p>

## 1-Introduction

Functional foods contain one or more compounds such as probiotics and prebiotics that have the ability to improve the health of consumers through non-nutritional mechanisms. Probiotics are defined as live microorganisms that, when consumed in sufficient quantities ( $10^6$  to  $10^9$  cfu/ml), are beneficial to the host. Probiotic microorganisms should be part of the normal microbial flora of the human intestine, large numbers of which survive the passage through the upper digestive tract and have beneficial effects in the intestine [1]. Lactic acid bacteria (LAB) must have certain criteria to be selected as probiotic bacteria for the production of probiotic cheese. These bacteria must originate from the human intestine, have the ability to tolerate acids and bile salts, and be compatible with the technological processes of cheese production [2,3].

Previous research has shown that the bacterium *Lactobacillus acidophilus* has probiotic properties and can grow in the presence of various prebiotics. Prebiotics are non-digestible food compounds that stimulate the growth of one or more health-promoting bacteria in the large intestine. Various types of carbohydrates, such as inulin, fructans, transgalactooligosaccharides, and lactulose, are prebiotics. Synbiotic foods contain probiotic bacteria and prebiotics [4,5].

Ricotta cheese is a fresh cheese produced by acidifying and heating whey from the cheesemaking process. Ricotta cheese is a high-moisture cheese with a pH of about 6, so it has a limited shelf life and is susceptible to microbial spoilage. This type of cheese has a compact structure, granular texture, a pleasant taste, and a yellowish-white color. Ricotta cheese is the most famous traditional cheese produced by heating whey. The viscosity of ricotta cheese, as a physical and

textural characteristic of the cheese, and its color, as a visual appeal, have a key impact on consumer acceptance of the product [7,6].

Ricotta cheese production using a combination of whey and whole milk has been reported in previous studies [6]. However, changes in viscosity and color characteristics of probiotic ricotta cheese containing *Lactobacillus acidophilus* have not been reported in previous studies. In one report, the physicochemical properties of synbiotic ricotta cheese containing *Lactobacillus paracasei* have been evaluated [8]. Also, in one study, the physicochemical properties of probiotic ricotta cheese obtained from cow's milk have been investigated [9]. However, no study has been conducted so far on the effect of using inulin and calcium chloride salt on changes in viscosity and color characteristics of probiotic ricotta cheese, so this issue will be addressed in the current study.

## 2- Materials and methods

### 2-1- Materials

This research was conducted at Islamic Azad University, Tehran Branch (Research Laboratory of Food Science and Technology Engineering Department) and Tehran Pegah Company. For do this study, whey from cow's milk (6.82% total solids, 0.4% fat, and 1.03% protein) was purchased from Tehran Pegah Company. Long-chain inulin (degree of polymerization 23-25, purity  $\geq 99.8\%$ ) was provided from Beneo Company (Mannheim, Germany), calcium chloride, and edible citric acid were purchased from Tozhaway Company (Tehran, Iran). Lyophilized *Lactobacillus acidophilus* (LA-5) was prepared from Christian Hansen Company (Horschelm, Germany). All chemicals and microbial culture media used in this study were provided from Merck Company (Germany).

## 2-2- Methods

### 2-2-1- Probiotic bacteria confirmation tests

In order to ensure the probiotic properties of the prepared *Lactobacillus acidophilus* (LA-5), the morphology of colonies grown on MRS agar medium containing salicin (0.5%) was examined by microscopic observation and the Gram test was performed on the samples. In addition, acid resistance, heat resistance, bile salt resistance, catalase and sugar fermentation tests were performed on the samples [11,10].

### 2-2-2- Synbiotic ricotta cheese production

Sweet whey produced from a traditional cheese production line was collected, 0.1% (w/v) salt and calcium chloride (0, 150 and 300 mg/L) were added to it. The resulting mixture was heated to 90°C and citric acid solution (0.11 mg/L) was added in a cheese making vat. Subsequently, the protein in the whey coagulated and formed ricotta cheese curds, were separated from the whey by a cloth filter and stored for 30 min. Then, by adding inulin (0%, 1% and 3%) and LA-5 ( $10^7$  cfu/g), the ricotta cheese was homogenized at 500 rpm for 5 min (Wigens, Germany). Subsequently, the ricotta cheese samples were incubated at 37°C for 60 minutes in an incubator (Memmert, Germany) and stored in sterile glass jars with lids at 4°C for 5 days in the refrigerator [8].

### 2-2-3- Measuring viscosity with a rheometer

Rheometric properties such as viscosity of ricotta cheese samples were measured using a rheometer (MCR52, Anton Paar, Germany) with shear stress control capability. The rheometer geometry was a parallel plate type and the distance between the rheometer and the plate below it was set to about 4 mm and

V 3.61 software was used. The viscosity of the cheese samples was evaluated on the 5<sup>th</sup> day of storage. In order to measure the viscosity as a function of shear rate and determine the flow behavior of the samples, the shear rate was used in the range of 0–300 s at 4°C. The test data were fitted with the power model (Equation 1) to determine the most appropriate model to describe the flow behavior [12]:

$$\text{Equation(1)} \quad \tau = k \dot{\gamma}^n$$

### 2-2-4- Measuring of color index

The color of ricotta cheese samples was evaluated using a Hunterlab colorimeter (UltraScanvis, USA). The basis of colorimetry in this system was the measurement of  $b^*$  (yellow-blue index),  $a^*$  (red-green index), and  $L^*$  (lightness index) index [13,14].

### 2-2-5- Evaluation of *Lactobacillus acidophilus* (LA-5) viability

To enumerate *Lactobacillus acidophilus* (LA-5) bacteria, serial dilutions ( $10^{-1}$ - $10^{-10}$ ) were prepared from ricotta cheese samples (10 g) using physiological serum and the samples were cultured in MRS agar medium containing salicin (0.5%) using the pour plate method. The plates were incubated for 72 hours in a CO<sub>2</sub> incubator at 37°C and the counting results were reported as Log cfu/ml [16,15].

### 2-2-6- Statistical analysis

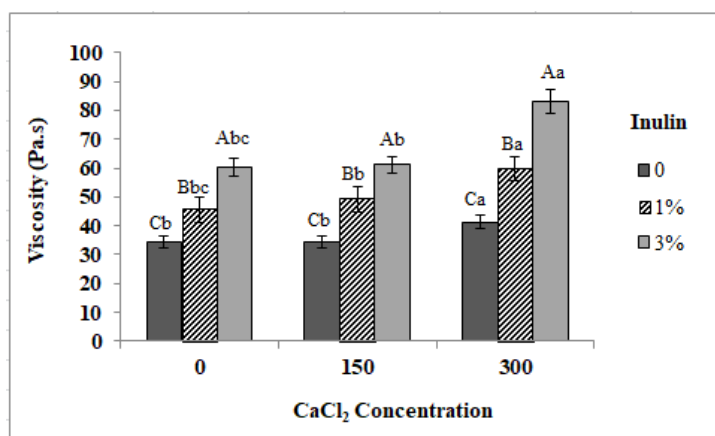
In this study, the effect of adding inulin and calcium chloride salt was investigated based on a completely randomized three-factor factorial design with three replications. Analysis of variance and comparison of means were performed using ANOVA and Duncan's multiple range test at the 95% probability level using SPSS software (version 22), and graphs were drawn using Excel 2010 software.

### 3- Results and discussion

#### 3-1- Viscosity evaluation in synbiotic ricotta cheese samples

Figure 1 shows the changes in apparent viscosity in different samples of synbiotic ricotta cheese on the 5<sup>th</sup> day of storage. The results showed that with increasing inulin concentration, the viscosity of synbiotic ricotta cheese samples increased significantly ( $p < 0.05$ ). Also, enrichment of ricotta cheese samples with calcium chloride salt caused a significant increase in the viscosity of cheese samples ( $p < 0.05$ ). The synbiotic ricotta cheese sample containing 3% inulin and 300 mg/L calcium chloride had the highest viscosity and the control sample had the lowest viscosity. Viscosity is one of the most

important textural properties of ricotta cheese, and a desirable viscosity increases the rubability of the cheese and makes it more desirable to eat. According to the results, the viscosity of the synbiotic ricotta cheese samples increased significantly with increasing the concentration of inulin and calcium chloride salt. It seems that when calcium chloride salt is added to the milk used in cheese preparation, it stabilizes and strengthens the gel structure of the cheese. The presence of calcium chloride salt helps to form a more cohesive protein network and increases the viscosity of the cheese by trapping more water and fat [17].



**Fig 1.** The effect of inulin and CaCl<sub>2</sub> on viscosity of synbiotic ricotta cheese during storage

<sup>a</sup>Means followed by different letters (a–d) show significant different ( $p < 0.05$ ) between treatments with same inulin concentration

<sup>b</sup>Means followed by different letters (A–D) show significant different ( $p < 0.05$ ) between treatments with same CaCl<sub>2</sub> concentration

In a study on the effect of different salts on the rheological properties of cheese, it was reported that calcium salt affects the viscosity and firmness of curd, and higher calcium concentrations lead to more whey being released from the curd, increasing the viscosity and firmness of the curd [18]. In a report on the effect of calcium chloride salt on the coagulation of milk protein and the resulting curd, it was observed that the

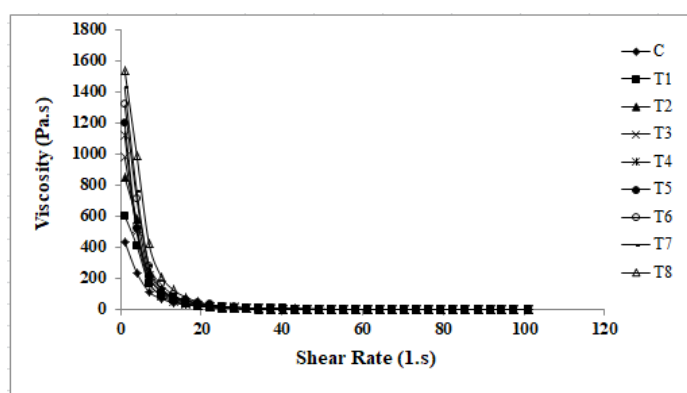
viscosity of cheese curd increases with increasing the concentration of calcium chloride salt [19].

#### 3-2- Apparent viscosity-shear rate evaluation of synbiotic ricotta cheese samples

Figure 2 is the flow chart of the synbiotic ricotta cheese samples and shows the relationship between apparent viscosity and

shear rate in the cheese samples. According to the graph, the apparent viscosity of all synbiotic ricotta cheese samples decreased with increasing shear rate; therefore, the cheese samples are considered non-Newtonian foods. At low shear rates, the difference between the apparent viscosity of the treated and control samples was dependent on the concentration of inulin and

calcium chloride salt. Samples containing higher concentrations of inulin and calcium chloride salt showed a greater apparent viscosity difference from the control sample. However, at high shear rates, the difference between the apparent viscosity of the treated and control samples decreased.



**Fig 2.** The flow curve of synbiotic ricotta cheese samples (C(0%Inulin, 0mg/L CaCl<sub>2</sub>), T<sub>1</sub>(0%Inulin, 150mg/L CaCl<sub>2</sub>), T<sub>2</sub>(0%Inulin, 300mg/L CaCl<sub>2</sub>), T<sub>3</sub>(1%Inulin, 0mg/L CaCl<sub>2</sub>), T<sub>4</sub>(1%Inulin, 150mg/L CaCl<sub>2</sub>), T<sub>5</sub>(1%Inulin, 300mg/L CaCl<sub>2</sub>), T<sub>6</sub>(3%Inulin, 0mg/L CaCl<sub>2</sub>), T<sub>7</sub>(3%Inulin, 150mg/L CaCl<sub>2</sub>), T<sub>8</sub>(3%Inulin, 300mg/L CaCl<sub>2</sub>))

According to the results, the control and treated cheese samples are non-Newtonian food materials in terms of rheological properties and show shear thinning behavior. In foods with shear thinning behavior, viscosity decreases with increasing shear rate. The intensity of the relationship between viscosity and shear rate depends on the concentration of the constituent components and the product temperature. The reason for the shear thinning behavior in synbiotic ricotta cheese is the rearrangement of food particles with increasing shear rate, which causes resistance to flow and a decrease in viscosity [20]. In the control ricotta cheese sample with a small flow index (n), there was a sudden decrease in viscosity at low shear rates due to the reduction in particle size under the influence of applied forces. In the

treated ricotta cheese samples containing inulin and calcium chloride salt, due to electrostatic interactions between milk proteins and calcium chloride salt and inulin, the flow index (n) was larger and more resistant to low shear rates [21]. In a report on the effect of hydrocolloids on the rheological properties of processed cheese, a significant increase in the viscosity of cheese samples was reported in the presence of agar, kappa carrageenan, and gelatin hydrocolloids [22]. In a study on the rheological properties of mozzarella cheese, shear-thinning behavior was observed with increasing shear rate [20].

### 3-3- Rheological model evaluation of in synbiotic ricotta cheese samples

Table 1 shows the rheological parameters obtained from the power model of different samples of synbiotic ricotta cheese on day 5

of storage. According to the results of the table, all samples showed pseudoplastic behavior within the range of shear rates used. For pseudoplastic food, the flow behavior index indicates a deviation from the

Newtonian behavior of the food, and as the flow behavior index moves away from 1, the behavior of the material changes from Newtonian to non-Newtonian behavior.

**Table1.** Parameters of Power law model of synbiotic ricotta cheese samples

$t = k(\dot{\gamma})^n$ Power law					
Samples	Inulin	CaCl <sub>2</sub> (mg/L)	K(Pa.s)	N	R <sup>2</sup>
C	0	0	2196	0.98	0.966
T <sub>1</sub>	0	150	2330	0.95	0.984
T <sub>2</sub>	0	300	2557	0.94	0.958
T <sub>3</sub>	1%	0	3072	0.86	0.984
T <sub>4</sub>	1%	150	3230	0.85	0.973
T <sub>5</sub>	1%	300	3457	0.84	0.934
T <sub>6</sub>	3%	0	3777	0.73	0.988
T <sub>7</sub>	3%	150	4115	0.65	0.953
T <sub>8</sub>	3%	300	4318	0.52	0.941

The highest flow index was for the control sample, and the flow behavior index decreased with increasing calcium chloride concentration. Also, increasing inulin concentration also caused a decrease in the flow index, and this decrease in the flow behavior index reached its highest value in samples containing calcium chloride and inulin, which confirms the increase in pseudoplastic behavior of cheese samples. According to the results, the consistency index increased with increasing inulin and calcium chloride salt concentrations, and the control sample had the lowest consistency index, and the synbiotic ricotta cheese sample containing 3% inulin and 300 mg/L calcium chloride had the highest consistency index.

According to the results, the rheological behavior of the synbiotic ricotta cheese samples follows the power model and in all samples it has a high correlation coefficient with the power model. In all samples, the flow index was less than 1, so the synbiotic

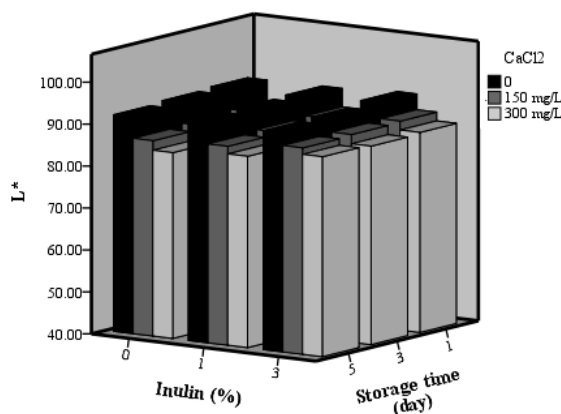
ricotta cheese samples had non-Newtonian behavior and the consistency coefficient increased with increasing inulin and calcium chloride salt concentrations. It seems that with increasing calcium chloride salt concentrations, electrostatic interactions between protein molecules increase and contribute to a more cohesive cheese texture. Inulin also has a thickening role as a hydrocolloid compound by absorbing water and contributes to increasing viscosity [6, 19]. Similarly, in a study on the rheological properties of mozzarella cheese, the power model was reported as a suitable rheological model for evaluating the rheological behavior of mozzarella cheese [23]. Also, in a report evaluating the rheological properties of fresco cheese, molar power was used to fit rheometer data [24].

### 3-4- Color indexes evaluation of synbiotic ricotta cheese samples

Figure 3 shows the changes in the L\* color index in different samples of synbiotic ricotta cheese during the storage period. Adding

inulin hydrocolloid to synbiotic ricotta cheese samples significantly increased the  $L^*$  color index of synbiotic ricotta cheese samples ( $p < 0.05$ ); samples containing more inulin were lighter than other samples. According to the results, with increasing calcium chloride concentration, the  $L^*$  color index of synbiotic ricotta cheese samples

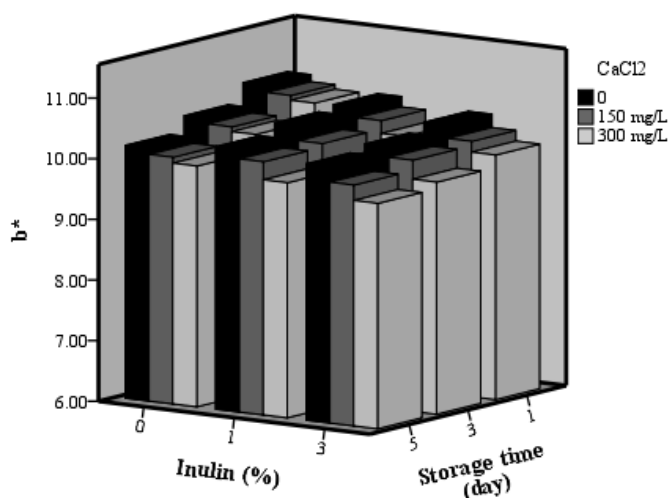
decreased significantly ( $p < 0.05$ ). Also, the  $L^*$  color index of synbiotic ricotta cheese samples decreased significantly during the 5-day storage period ( $p < 0.05$ ). According to the results, the synbiotic ricotta cheese samples containing 3% inulin and 0 mg/L calcium chloride salt had the highest  $L^*$  color index on the first day of storage.



**Fig 3.** The effect of inulin and  $\text{CaCl}_2$  on  $L^*$  color indexes of synbiotic ricotta cheese during storage (C(0%Inulin, 0mg/L  $\text{CaCl}_2$ ), T<sub>1</sub>(0%Inulin, 150mg/L  $\text{CaCl}_2$ ), T<sub>2</sub>(0%Inulin, 300mg/L  $\text{CaCl}_2$ ), T<sub>3</sub>(1%Inulin, 0mg/L  $\text{CaCl}_2$ ), T<sub>4</sub>(1%Inulin, 150mg/L  $\text{CaCl}_2$ ), T<sub>5</sub>(1%Inulin, 300mg/L  $\text{CaCl}_2$ ), T<sub>6</sub>(3%Inulin, 0mg/L  $\text{CaCl}_2$ ), T<sub>7</sub>(3%Inulin, 150mg/L  $\text{CaCl}_2$ ), T<sub>8</sub>(3%Inulin, 300mg/L  $\text{CaCl}_2$ ))

Figure 4 shows the changes in the color index  $b^*$  in different samples of synbiotic ricotta cheese during the storage period. According to the results, the color index  $b^*$  of different samples of synbiotic ricotta cheese decreased significantly during the storage period ( $p < 0.05$ ). In addition, increasing the concentration of inulin in different samples of synbiotic ricotta cheese was associated with a significant decrease in the color index  $b^*$  in the cheese samples ( $p < 0.05$ ). Synbiotic cheese samples without inulin had a higher

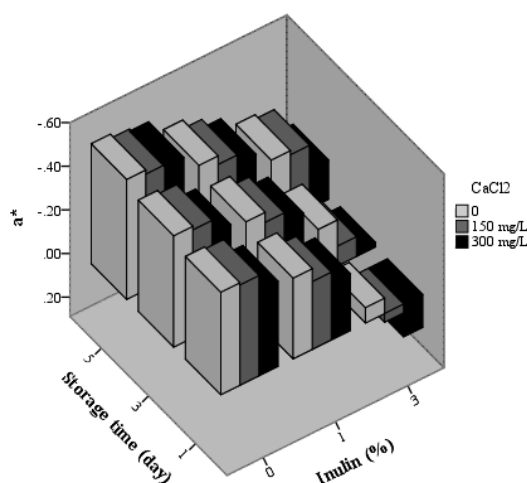
color index  $b^*$  compared to cheese samples containing 3% inulin. Also, increasing the concentration of calcium chloride salt, even at a concentration of 150 mg/L, significantly reduced the color index  $b^*$  in synbiotic ricotta cheese samples ( $p < 0.05$ ). According to the results, the control sample had the highest value of the color index  $b^*$  on the first day of storage, and the treatment sample containing 3% inulin and 300 mg/L calcium chloride on the 5th day of storage had the lowest value of the color index  $b^*$ .



**Fig 4.** The effect of inulin and  $\text{CaCl}_2$  on  $b^*$  color indexes of synbiotic ricotta cheese during storage (C(0%Inulin, 0mg/L  $\text{CaCl}_2$ ), T<sub>1</sub>(0%Inulin, 150mg/L  $\text{CaCl}_2$ ), T<sub>2</sub>(0%Inulin, 300mg/L  $\text{CaCl}_2$ ), T<sub>3</sub>(1%Inulin, 0mg/L  $\text{CaCl}_2$ ), T<sub>4</sub>(1%Inulin, 150mg/L  $\text{CaCl}_2$ ), T<sub>5</sub>(1%Inulin, 300mg/L  $\text{CaCl}_2$ ), T<sub>6</sub>(3%Inulin, 0mg/L  $\text{CaCl}_2$ ), T<sub>7</sub>(3%Inulin, 150mg/L  $\text{CaCl}_2$ ), T<sub>8</sub>(3%Inulin, 300mg/L  $\text{CaCl}_2))$

Figure 5 shows the changes in  $a^*$  color index in different samples of synbiotic ricotta cheese during the storage period. Adding inulin hydrocolloid to synbiotic ricotta cheese samples significantly increased  $a^*$  color index of synbiotic ricotta cheese samples as a negative color index ( $p < 0.05$ ); samples containing inulin were more red than other samples. Also, with increasing calcium chloride concentration,  $a^*$  color index of

different synbiotic ricotta cheese samples increased significantly ( $p < 0.05$ ). The treatment sample containing 3% inulin and 300 mg/L calcium chloride had the highest  $a^*$  color index on the first day of storage; while the lowest  $a^*$  color index was assigned to the control sample. Also, during the storage period,  $a^*$  color index of synbiotic ricotta cheese samples decreased and with the progress of storage time,  $a^*$  color index became more negative.



**Fig 5.** The effect of inulin and  $\text{CaCl}_2$  on  $a^*$  color indexes of synbiotic ricotta cheese during storage(C(0%Inulin, 0mg/L  $\text{CaCl}_2$ ),  $T_1$ (0%Inulin, 150mg/L  $\text{CaCl}_2$ ),  $T_2$ (0%Inulin, 300mg/L  $\text{CaCl}_2$ ),  $T_3$ (1%Inulin, 0mg/L  $\text{CaCl}_2$ ),  $T_4$ (1%Inulin, 150mg/L  $\text{CaCl}_2$ ),  $T_5$ (1%Inulin, 300mg/L  $\text{CaCl}_2$ ),  $T_6$ (3%Inulin, 0mg/L  $\text{CaCl}_2$ ),  $T_7$ (3%Inulin, 150mg/L  $\text{CaCl}_2$ ),  $T_8$ (3%Inulin, 300mg/L  $\text{CaCl}_2$ ))

The color of ricotta cheese is one of the physical characteristics that influence the choice of the product by consumers. An increase in the color index  $L^*$  indicates an increase in brightness and a decrease indicates an increase in darkness in the food. According to the results, with an increase in the concentration of inulin in the synbiotic ricotta cheese samples, the color index  $L^*$  in the cheese samples increased. It is likely that inulin, by binding to reducing sugars in the cheese, prevents their reaction with amino acids and Maillard interaction and the formation of brown pigments, and as a result, the brightness of the synbiotic ricotta cheese increases [25]. In addition, increasing the concentration of calcium chloride salt caused a decrease in the brightness of the cheese samples. It is likely that increasing the concentration of calcium chloride salt affects the cross-links between casein micelles and causes a change in the  $L^*$  color index of the cheese samples. Also, the addition of calcium salt causes a change in the mineral content of the cheese, which affects the  $L^*$  color index of the cheese samples. According to the results, the  $L^*$  color index of the synbiotic ricotta cheese samples decreased significantly during the storage period, and this decrease seems to be due to the increase in the intensity of proteolysis and lipolysis, changes in the structure of protein and fat, and color compounds during storage [26]. Similarly, in a study on processed analog cheese, an increase in the  $L^*$  color index of cheese samples was reported with increasing inulin concentration, and the reason for this increase was attributed to the effect of inulin hydrocolloid on light scattering [27]. Also, in a study on the effect of sodium and calcium salts on the physicochemical properties of

feta cheese, a decrease in the  $L^*$  color index of cheese samples was reported with increasing calcium chloride salt concentration [21].

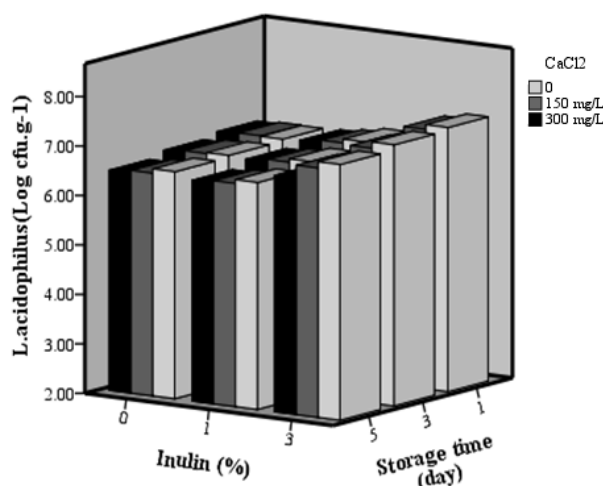
According to the results, the  $b^*$  color index of the synbiotic ricotta cheese samples ranged from 9.7 to 10.76. Similarly, Recosan cheese produced from whey also showed a  $b^*$  color index between 9.1 and 14.81 [27]. It seems that the significant decrease in  $b^*$  color index during storage is due to oxidation and hydrolysis of carotenoid pigments in the cheese samples. In addition, increasing inulin concentration was associated with a decrease in  $b^*$  color index in the cheese samples, which is probably due to the formation of complexes with pigments and the prevention of the Maillard reaction [29]. Similarly, in a report on the effect of replacing inulin with fat in analog cheese, it was found that the  $b^*$  color index decreased in cheese samples containing inulin, and there was a positive relationship between inulin concentration and the decrease in  $b^*$  color index. Contrary to the results of a recent study on the physicochemical properties of Swiss kefir cheese containing inulin, a non-significant decrease in the color index  $b^*$  was observed in cheese samples [29]. According to the results, a significant decrease in the color index  $b^*$  in cheese samples with increasing calcium chloride concentration may be due to the reaction of calcium salt with yellow pigments or the effect on the interaction of carotenoids with other compounds effective in creating color [26]. In a report on the effect of calcium salt on the physicochemical properties of goat milk cheese, a decrease in the intensity of the yellow color was observed with increasing calcium chloride salt concentration [30].

The  $a^*$  color index in cheese samples indicates red color and is a negative color index, and the intensity of the red color increases with increasing the  $a^*$  color index. According to the results, with increasing inulin concentration,  $a^*$  color index of ricotta cheese samples increases and the color of the samples changes from green to red. It seems that inulin increases the water absorption capacity of ricotta cheese samples and acts as a color intensifier, improving the color of the cheese samples [27]. In a recent study,  $a^*$  color index of all samples was in the range of -0.15 to -0.55. Similarly, in a study evaluating the physicochemical properties of ricotta cheese enriched with hydrocolloids,  $a^*$  color index of all samples was reported to be in the range of -0.14 to -0.42. According to the results, the intensity of the  $a^*$  color index of synbiotic ricotta cheese samples decreased during the storage period. It is likely that oxidation and degradation of the pigments that cause the red color in ricotta cheese samples during storage causes a decrease in the intensity of the red color [26]. Also, in a report investigating the physicochemical properties of Swiss kefir cheese containing inulin, a significant decrease in  $a^*$  color index was observed during storage [29]. Similarly, in a study investigating the physicochemical properties

of ricotta cheese, a decreasing trend in  $a^*$  color index was reported during storage [31].

### 3-5- Determination of *Lactobacillus acidophilus* (LA-5) viability in synbiotic ricotta cheese samples

Figure 6 shows the changes in the survival of *Lactobacillus acidophilus* in synbiotic ricotta cheese samples during storage. According to the results, adding inulin hydrocolloid to synbiotic ricotta cheese samples significantly increased the survival of *Lactobacillus acidophilus* in synbiotic ricotta cheese samples ( $p < 0.05$ ); samples containing more inulin contained a higher number of *Lactobacillus acidophilus* bacteria than other samples. According to the results, with increasing calcium chloride concentration, the survival of *Lactobacillus acidophilus* in synbiotic ricotta cheese samples significantly increased ( $p < 0.05$ ). Meanwhile, the survival of *Lactobacillus acidophilus* in synbiotic ricotta cheese samples decreased significantly during the 5-day storage period ( $p < 0.05$ ). According to the results, on the fifth day of storage, synbiotic ricotta cheese samples containing 3% inulin and 300 mg/L of calcium chloride salt had the highest survival of *Lactobacillus acidophilus*.



**Fig 6.** The effect of inulin and CaCl<sub>2</sub> on *L. acidophilus* LA-5 Survival (CFU/g) in synbiotic ricotta cheese (C(0%Inulin, 0mg/L CaCl<sub>2</sub>), T<sub>1</sub>(0%Inulin, 150mg/L CaCl<sub>2</sub>), T<sub>2</sub>(0%Inulin, 300mg/L CaCl<sub>2</sub>), T<sub>3</sub>(1%Inulin, 0mg/L CaCl<sub>2</sub>), T<sub>4</sub>(1%Inulin, 150mg/L CaCl<sub>2</sub>), T<sub>5</sub>(1%Inulin, 300mg/L CaCl<sub>2</sub>), T<sub>6</sub>(3%Inulin, 0mg/L CaCl<sub>2</sub>), T<sub>7</sub>(3%Inulin, 150mg/L CaCl<sub>2</sub>), T<sub>8</sub>(3%Inulin, 300mg/L CaCl<sub>2</sub>))

According to the Iranian national standard, the minimum amount of probiotic microorganisms in food to benefit from health benefits is 6 Log cfu/g of food. According to the results, the survival rate of *Lactobacillus acidophilus* bacteria in synbiotic ricotta cheese samples is in the range of Log cfu/g 6.34-7.41, so all ricotta cheese samples have the standard level of probiotic bacteria during the storage period, and due to the presence of inulin as a prebiotic substance, all ricotta cheese samples are synbiotic foods [31]. According to the results, the survival of *Lactobacillus acidophilus* bacteria increased in the presence of inulin and calcium chloride salt and decreased significantly during storage. According to previous reports, decreased pH, production of organic acids, high redox potential, production of hydrogen peroxide, presence of molecular oxygen, bacterial competition and temperature changes during storage reduce the viability of probiotic bacteria during storage [3]. Inulin as a prebiotic compound has a stimulating effect on the growth of probiotic bacteria [21]. Similarly, in a study on the effect of calcium salts on the characteristics of feta cheese, calcium salts were reported to increase the growth of starter bacteria in cheese samples [32]. In a study on the physicochemical properties of probiotic mascarpone cheese, inulin was found to improve the growth of *Bifidobacterium animalis* in cheese samples [33].

#### 4-Conclusion

Cheese is one of the most important foods used as a carrier of probiotic health-promoting bacteria. Considering the high amount of whey produced in the country's

dairy factories, the production of ricotta cheese from whey is a suitable solution for the optimal use of whey produced as waste from dairy factories. The production of synbiotic ricotta cheese creates added value in the produced whey and also leads to the improvement of the health of consumers. In this study, changes in the physical properties of synbiotic ricotta cheese in the presence of inulin and calcium chloride salt were investigated. The results of this study showed a significant increase in the survival of *Lactobacillus acidophilus* in synbiotic ricotta cheese in the presence of inulin and calcium chloride salt. According to the results, the control and treated cheese samples were non-Newtonian food materials in terms of rheological properties and showed shear thinning behavior. With increasing concentrations of inulin and calcium chloride salt, the viscosity of synbiotic ricotta cheese samples increased, which is due to the water absorption capacity of inulin hydrocolloid and the essential role of calcium in the protein gel structure of cheese. According to the results, the addition of inulin hydrocolloid and calcium chloride salt caused a decrease in the brightness and intensification of yellowness and redness in cheese samples. In general, the results of the recent study recommend the production of ricotta cheese from whey, which is a waste product of dairy industries, for the production of functional food and the optimal use of whey.

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مقاله علمی-پژوهشی

### بررسی تاثیر اینولین و کلرید کلسیم بر ویسکوزیته و ویژگی های رنگی پنیر ریکوتا سین بیوتیک

مرجانه صدقاتی<sup>\*</sup>، ریحانه کاوند

۱-عضو هیات علمی گروه علوم و صنایع غذایی، دانشکده علوم زیستی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران

۲-دانشجوی کارشناسی ارشد گروه علوم و صنایع غذایی، دانشکده علوم زیستی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران

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#### کلمات کلیدی:

اینولین،

پنیر،

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شاخص رنگی،

ویسکوزیته.

این تحقیق با هدف بررسی تغییرات ویسکوزیته و خصوصیات رنگی پنیر ریکوتا سین بیوتیک با افزودن اینولین و نمک کلرید کلسیم در طول دوره نگهداری انجام شد. برای تولید پنیر ریکوتا سین بیوتیک از آب پنیر، لاکتوباسیلوس/اسیدوفیلوس، اینولین و کلرید کلسیم استفاده شد. بدین منظور از اینولین در سه سطح ۰، ۱٪ و ۳٪ و نمک کلرور کلسیم در سه سطح ۰، ۱۵۰ و ۳۰۰ میلی گرم در لیتر استفاده گردید. تغییرات ویسکوزیته و تغییرات رنگی (شاخص های رنگی  $L^*$ ،  $b^*$  و  $a^*$ ) و زنده مانگی باکتری لاکتوباسیلوس/اسیدوفیلوس در طول دوره نگهداری بررسی شد. طبق نتایج افزودن اینولین و نمک کلرور کلسیم سبب افزایش معنی دار ویسکوزیته پنیر ریکوتا سین بیوتیک شد ( $p < 0/05$ ). نمودار ویسکوزیته ظاهری-سرعت برشی مشخص کرد با افزایش سرعت برشی ویسکوزیته ظاهری نمونه ها کاهش یافت؛ بنابراین نمونه های پنیر ریکوتا سین بیوتیک جز مواد غذایی غیر نیوتنی هستند. در ارزیابی پارامترهای رئولوژیکی پنیر ریکوتا سین بیوتیک نمونه های پنیر همبستگی بالایی با مدل توان داشته و پنیر ریکوتا سین بیوتیک مورد استفاده رفتار سودوپلاستیک نشان دادند. تغییرات شاخص رنگی  $L^*$  را در پنیر ریکوتا سین بیوتیک نشان داد افزودن هیدروکلوئید اینولین به نمونه های پنیر شاخص رنگی  $L^*$  را به صورت معنی داری افزایش و حضور کلرور کلسیم شاخص رنگی  $L^*$  نمونه های پنیر ریکوتا سین بیوتیک را به صورت معنی داری کاهش داد ( $p < 0/05$ ). همچنین، افزایش غلظت اینولین و نمک کلرور کلسیم در نمونه های مختلف پنیر ریکوتا سین بیوتیک با کاهش معنی دار شاخص رنگی  $b^*$  و  $a^*$  در نمونه های پنیر همراه بود ( $p < 0/05$ ). طبق نتایج حاصل افزودن هیدروکلوئید اینولین و نمک کلرور کلسیم سبب بهبود زنده مانگی لاکتوباسیلوس/اسیدوفیلوس شد ( $p < 0/05$ ). با توجه به نتایج حاصل در نمونه پنیر ریکوتا سین بیوتیک حاوی ۳٪ اینولین و ۳۰۰ mg/L کلرور کلسیم ویسکوزیته بهبود یافته و خصوصیات رنگی نمونه مطلوب بود. ضمناً این نمونه بیشترین زنده مانگی لاکتوباسیلوس/اسیدوفیلوس را در بین نمونه های پنیر ریکوتا سین بیوتیک دارا بود.

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<sup>\*</sup> مسئول مکاتبات:

marjanehsedaghati@yahoo.com