

Annual Research & Review in Biology
4(24): 4413-4425, 2014

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Influence of Resistant Starch and Exopolysaccharide-Producing *Streptococcus thermophilus* on Viability of Lactic Acid Bacteria in low fat UF Feta Cheese during Ripening

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Authors' contributions

This work was carried out in collaboration among all authors. Author FST designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EH and MH supervised the searches and managed the analyses of the study. Author EE managed the literature searches and edited the manuscript analytically. All authors read and approved the final manuscript.

Original Research Article

Received 30th May 2014
Accepted 2nd August 2014
Published 14th August 2014

ABSTRACT

Aims: Lactic acid bacteria (LAB) are essential component of all natural cheese varieties, and play important roles during both cheeses producing and ripening. The objective of this study was to evaluate the effect of the combination of Exopolysaccharide-Producing strains and resistant starch on viability of lactic acid bacteria in low fat ultrafiltered (UF) Feta cheese over 60 days of ripening period at 5°C.

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Study Design: The cheesemaking experiment was carried out in triplicate using a complete randomized design.

Place and Duration of Study: The study was conducted in the department of Arjan Dairy Company (Research Development and Central Laboratory) and department of Food Science and Technology, Shahrekord Branch, Islamic Azad University (Laboratory of Food Science and Technology), between April, 2013 and January, 2014.

Methodology: Cheeses were manufactured with added EPS-producing strains and resistant starch individually or in combination. During ripening period determination of acidity%, pH values, moisture%, fat%, viable bacterial count and sensory evaluation were performed. Low-fat control treatment was containing 3% inulin and high-fat treatment was produced without any additive.

Results: The data indicated that the acidity and count of LAB strains in cheeses made with both EPS⁺ and different concentrations of resistant starch were higher than the cheeses made without EPS⁺ culture and with the different concentrations of resistant starch during cheese storage. Also these treatments had the highest moisture contents and sensorial scores compared with the cheeses made without EPS⁺ and with the different concentrations of resistant starch ($P < 0.05$).

Conclusion: In conclusion, all results provide evidence that the combination of EPS producing starter and different concentrations of RS had beneficial effects on the viability of LAB and sensorial scores in low fat UF Feta cheese during ripening.

Keywords: Exopolysaccharide; resistant starch; UF Feta cheese; low fat; lactic acid bacteria.

ABBREVIATIONS

UF = Ultra Filtration; RS = Resistant Starch; EPS⁺ = exopolysaccharide producing starter culture; CHO = Usual starter culture cheesemaking (Choozeit Feta A).

1. INTRODUCTION

High dietary fat consumption has been shown to be associated with an increased risk of obesity, atherosclerosis, coronary heart disease and elevated blood pressure [1]. This fact has led to increased consumer demand for low fat foods, including cheeses. In cheese, the removal or reduction of fat adversely affects both its flavor and texture [2-4]; low-fat cheeses are usually identified as bland, firm, rubbery and defective in color [5]. Since the major defects of low fat UF Feta cheese are related to texture and body problems, it has been suggested to increase its moisture content beyond that of full-fat cheese to overcome these problems [6].

Increasing moisture content in low fat cheese could be obtained by modifying cheese processing procedures [7-9] or using emulsifiers and thickening agents [10,11]. The use of exopolysaccharide (EPS)-producing lactic acid bacteria could be a potential alternative for thickening agents to increase moisture content and improve texture attributes of low fat cheese.

Hassan (2008) suggested that EPS have the ability to bind water and increase the moisture in the nonfat substance without the need to modify the cheesemaking procedure [12]. Many strains of dairy Lactic acid bacteria synthesize extracellular polysaccharides. LAB are an essential component of all natural cheese varieties and play important roles in the texture

development during both cheese manufacturing and ripening. The EPS produced by LAB may have technological and health benefits in food products [13-16]. The in situ use of these generally recognized as safe, food-grade bacteria as functional starter cultures in fermented dairy products is preferred over the addition of thickeners for the food product [13]. These polymers may be assembled as capsular (CPS) polysaccharides that are tightly associated with the cell surface, or they may be liberated into the growth medium (i.e., "ropy" polysaccharide). The term EPS may be used to describe either type of extracellular polysaccharide [15]. Bacterial EPS can be composed of one type of sugar monomer (homopolysaccharide) or consist of several types of monomers (heteropolysaccharide). Well-known examples of LAB homopolysaccharides include dextrans and glucans produced by *Leuconostoc mesenteroides* and *Streptococcus mutans*, respectively. Heteropolysaccharides are synthesized by many LAB, including *Streptococcus thermophilus*, *Lactococcus lactis* and dairy *Lactobacillus* subsp. [17,18].

Resistant starch is a kind of fat replacer that was recently recognized as source of fiber, and is classified as a fiber component with partial or complete fermentation in the colon, producing various beneficial effects on health. RS also offers an exciting new potential as a food ingredient. Since RS almost entirely passes the small intestine; it can behave as a substrate for growth of the probiotic microorganisms and considered as prebiotic agent to improve the growth and survival of probiotic bacteria [19,20].

Based on these considerations, the aim of this study was to evaluate the effect of different concentrations of RS and EPS-producing strains either individually or in combination on the production of low-fat UF Feta cheese by investigating the kinetics of acidification, pH value, viability of lactic acid bacteria, moisture, fat content and sensory properties of samples over a 60 days of ripening period.

2. MATERIALS AND METHODS

2.1 Materials

Exopolysaccharide-producing starter culture *Streptococcus thermophilus* (FD-DVS-ST Body-2), acid producing starter culture for cheesemaking (FD-DVS R704) with combination of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*, were obtained from Chr. Hansens Dairy Cultures (Denmark). Usual starter culture cheesemaking (Choozeit Feta A) with combination of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus helveticus* which are not gas-producing bacteria, were obtained from Danisco (France). Resistant starch (Hi- maize 260) was obtained from Ingredion Company (Germany, Hamburg). Man Rogosa–Sharpe (MRS) agar (Merck, KGaA 64271 Darmstadt, Germany), M17 agar (Merck, Darmstadt, Germany), the rennet (Chr, Hansen Dairy Cultures, Denmark), raw cow's milk, equipment, and filtration moduli were provided by Arjan dairy company (Shiraz, Iran).

2.2 Methods

To investigate the effect of different concentration of resistant starch and EPS-producing starter culture on low fat UF Feta cheese, ten treatments were prepared and labeled as follows (Table 1).

Table 1. Treatments of feta cheese production and their labels

Label	Treatments
Blank CHO	Low fat cheese without RS, produced by usual starter culture (Choozeit Feta A).
0.25%RS+CHO	Low fat cheese produced by adding 0.25% RS and usual starter culture (Choozeit Feta A).
0.5% RS+ CHO	Low fat cheese produced by adding 0.5% RS and usual starter culture (Choozeit Feta A).
0.75%RS+ CHO	Low fat cheese produced by adding 0.75% RS and usual starter culture (Choozeit Feta A).
Blank EPS	Low fat cheese without RS, produced by EPS-producing starter culture (ST Body- 2+R704).
0.25%RS+EPS	Low fat cheese produced by adding 0.25% RS and EPS-producing starter culture (ST Body- 2+R704).
0.5% RS+ EPS	Low fat cheese produced by adding 0.5% RS and EPS-producing starter culture (ST Body- 2+R704).
0.75% RS+ EPS	Low fat cheese produced by adding 0.75% RS and EPS-producing starter culture (ST Body- 2+R704).
Control low fat	Low fat cheese produced by adding 3% inulin and usual starter culture (Choozeit Feta A).
Control full fat	Full fat cheese without RS, produced by usual starter culture (Choozeit Feta A).

2.2.1 UF cheese preparation

Cheese treatments were made in Arjan dairy company (Shiraz, Iran) according to UF cheese making method proposed by Tetra-Pak company [21] (adapted with some modifications by Karami et al.) [22] (Fig. 1). To produce full fat cheese, retentate contained 35% dry matter and 16% fat, and to produce low fat cheese, it contained 26% dry matter and 0.5% fat. The Ratio of inoculation was 40/60 (ST Body- 2/ R704). In the preripening stage (27°C), after decreasing the cheese pH to 4.70, cheese samples were transferred to the cold room (5°C) for cooling and ripening for 1 to 8 weeks. During this period determination of acidity, pH values, viable bacterial count, moisture, fat content and sensory characteristics of samples were performed.

2.2.2 Enumeration of viable starter bacteria

Media (MRS or M17) were prepared according to the manufacturer's instructions. Cheese samples (10 g) were homogenized for 30 min with 90mL of sterile 2% (W/V) sodium citrate solution in sterile stomacher bags. The resulting solution (1/10 dilution) was used to prepare further dilutions. Subsequent dilutions (10^{-2} to 10^{-8}) were prepared in sterile 0.1% (W/V) peptone water. Appropriately, 1mL of the diluted samples (10^{-7} and 10^{-8}) were then transferred by sampler to the molten media (MRS or M17) agar. After solidifying, and in order to create microaerophilic conditions, plates were inverted in anaerobic jar. The anaerobic jar was incubated at 37°C for 48h to determine the total viable bacterial count. Total viable cocci were enumerated on M17 agar medium, while MRS agar medium was

used for both viable lactobacilli and lactococci, and bacteria were counted by colony counter (Shimi Fan Co, Iran). Each colony represents a "Colony Forming Unit" (CFU) [23].

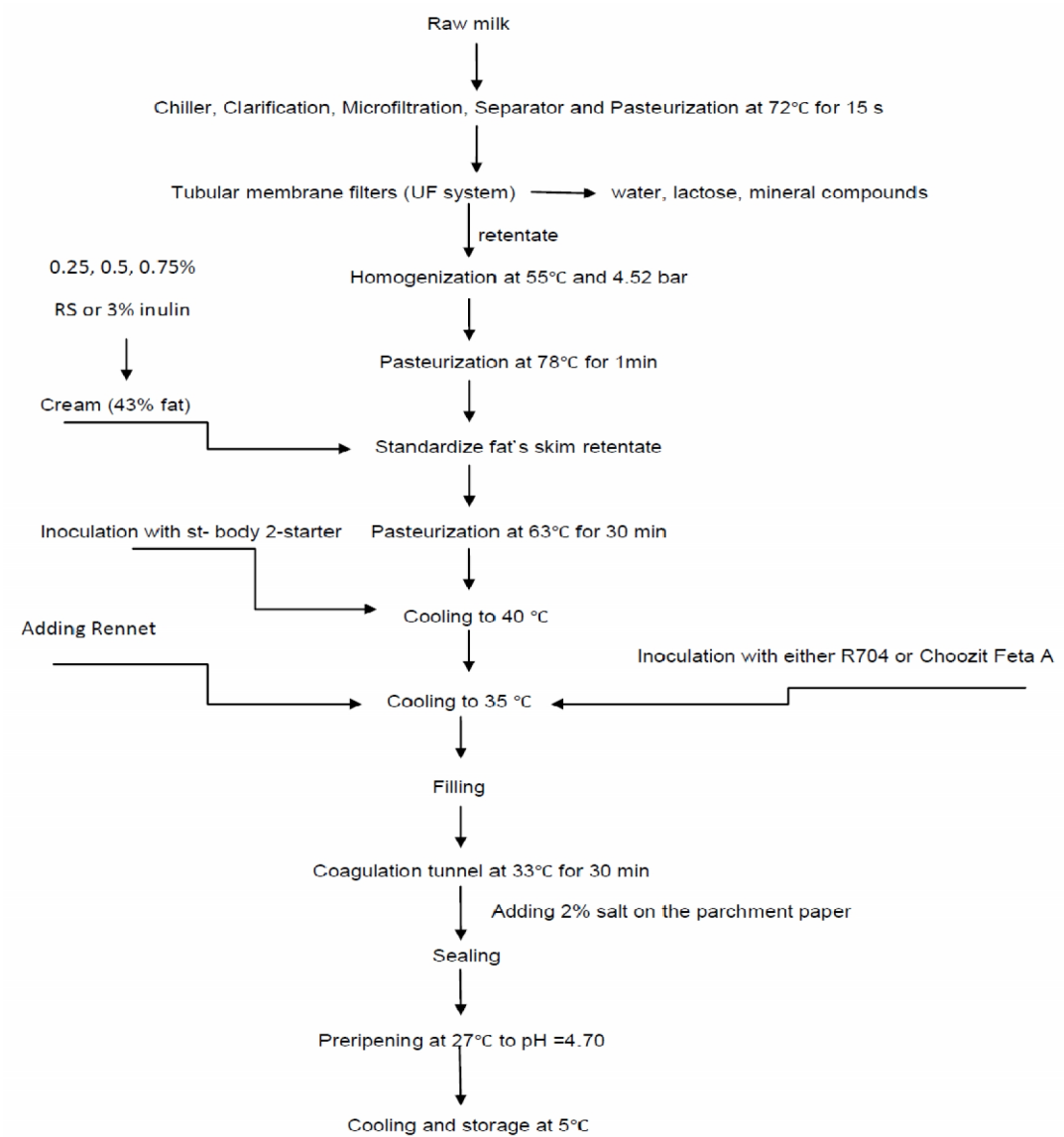


Fig. 1. Flowchart of cheese making

2.2.3 Compositional analysis of UF feta cheese during ripening

UF Feta cheese treatments were analyzed for moisture, fat, acidity and pH values during 60 days of ripening period. The moisture content in UF Feta cheese was determined by drying at 103±5°C till reaching a constant weight of the dried samples [24]. The fat content in cheese was determined by Gerber method [25]. The pH of the cheese was measured by

direct insertion of an electrode (pH-Meter 766-Calimatic (Knick), Germany) into grated cheese. Acidity in cheese was estimated by titration [26].

2.2.4 Sensory evaluation of Feta cheeses with different treatments

In this case, consumer oriented test was conducted by 20 volunteers, eighth week after production. The testing panel consisted of students from Azad University of Kazerun, Iran. Each panelist received 20 g of cube cheese samples that were placed into a clear cup labeled with random three digit number and evaluated on sensory characteristics such as the spread ability, odor, taste, texture, color and overall acceptability of each cheese sample on a 5-point hedonic scale (1= like extremely, 2= like very much, 3= like moderately, 4= like slightly and 5= neither like or dislike) [27].

2.2.5 Statistical analysis

The cheese making experiment was carried out in triplicate using a complete randomized design. Statistical calculation was performed using SPSS Statistical Software version 19, and the resulted values are presented as means \pm standard deviations. Evaluation of significance was performed by ANOVA and Duncan tests in significance levels of $P < 0.05$. The sensorial properties chart was drawn using Microsoft Office Excel 2007.

3. RESULTS AND DISCUSSION

3.1 Viable Bacterial Count

Lactic acid bacteria are essential component of all natural cheese varieties, and play important roles during both cheese producing and ripening. So, the enumeration of these bacteria for analyzing physico-chemical and sensorial properties of cheese seem to be important [28]. Bacterial enumeration on MRS medium was approximately the same as M17 medium during cheese ripening (Tables 2, 3). The results showed that the full fat and blank CHO treatments of starter had the highest and the lowest enumeration, respectively, on both MRS and M17 during storage. This finding is in agreement with the results of Laloy et al. [29] who reported that microbial population increases with increasing fat content in cheddar cheese. The data showed that the count of LAB strains in cheese treatments made with both EPS+ and different concentrations of resistant starch was higher than the cheeses made without EPS+ and with different concentrations of resistant starch during cheese storage ($P < 0.05$). This finding is consistent with the results of Perry et al. [30]. Probably, the sticky nature of the exopolysaccharides increases the viability of bacteria in the cheese during ripening period. The count of LAB in cheese samples with both RS and without EPS+ on M17 agar increased by increasing resistant starch concentration during ripening period, although such increase was not significant in some samples ($P > 0.05$). Also, Gustaw et al. [31] reported that resistant starch with high amylose content increases survival of lactic acid bacteria in yoghurt during storage [31]. The highest count of LAB on MRS and M17 agar were at the highest in the third week, and then the viability of LAB bacteria was decreased. The results showed that full fat and blank CHO treatments had the highest and the lowest enumeration, respectively, of viable LAB on both MRS and M17 during storage.

Table 2. Lactic acid bacteria (Log CFU/mL) on MRS media during storage of UF Feta cheeses with different treatments (mean ± standard deviation)

Time treatments	First week	Third week	Sixth week	Eighth week
Full fat	0.00 ^{Ca} 9.63	9.68±0.01 ^{Aa}	9.67±0.01 ^{Ba}	9.60±0.01 ^{Da}
Low fat	9.53±0.00 ^{Cb}	9.57±0.00 ^{Abc}	9.57±0.00 ^{Ab}	9.54±0.01 ^{Bb}
Blank with EPS	9.36±0.01 ^{Bg}	9.43±0.01 ^{Ae}	9.26±0.01 ^{Ct}	8.99±0.00 ^{Ug}
0.25%RS + EPS	9.51±0.01 ^{Cc}	9.58±0.01 ^{Ab}	9.56±0.01 ^{Bb}	9.49±0.01 ^{Lc}
0.5%RS + EPS	9.47±0.00 ^{Ce}	9.56±0.00 ^{Ac}	9.53±0.00 ^{Bc}	9.46±0.01 ^{Dd}
0.75%RS + EPS	9.49± ^{Cd} 0.00	9.57± ^{Abc} 0.00	9.56±0.00 ^{Bb}	9.49± ^{Cc} 0.01
BlankCHO	9.08± ^{Bi} 0.01	9.22± ^{At} 0.03	8.84± ^{Cg} 0.03	8.80±0.01 ^{Ch}
0.25%RS + CHO	9.37± ^{Bf} 0.01	9.45± ^{Ae} 0.01	9.44±0.01 ^{Ae}	9.36±0.01 ^{Be}
0.5%RS + CHO	9.34±0.00 ^{Ch}	9.44± ^{Ae} 0.01	9.43±0.00 ^{Be}	9.30±0.01 ^{Lt}
0.75%RS + CHO	9.47±0.00 ^{Ce}	9.53± ^{Ad} 0.00	9.49±0.01 ^{Bd}	9.47± ^{Cd} 0.01

* Small shared letters indicate no significant differences in each column and large shared letters indicate no significant difference in each row

Table 3. Lactic acid bacteria (Log CFU/mL) on M17 media during storage of UF Feta cheeses with different treatments (mean ± standard deviation)

Time Treatments	First week	Third week	Sixth week	Eighth week
Full fat	9.68± 0.00 ^{Ba*}	9.74±0.01 ^{Aa}	9.67±0.01 ^{Ba}	9.64±0.00 ^{Ca}
Low fat	9.57±0.02 ^{Cb}	9.62±0.01 ^{Ab}	9.59±0.03 ^{Bb}	9.55±0.01 ^{Db}
Blank with EPS	9.41±0.01 ^{Bg}	9.45±0.01 ^{Ad}	9.38±0.02 ^{Cg}	9.04±0.00 ^{Dh}
0.25%RS + EPS	9.51±0.01 ^{Cd}	9.62±0.01 ^{Ab}	9.59±0.02 ^{Bb}	9.53±0.01 ^{Lb}
0.5%RS + EPS	9.53±0.03 ^{Cc}	9.60±0.00 ^{Ab}	9.57±0.03 ^{Bc}	9.49±0.01 ^{Dd}
0.75%RS + EPS	9.54± ^{Cc} 0.00	9.61± ^{Ab} 0.00	9.59±0.00 ^{Bb}	9.51± ^{Lc} 0.01
Blank CHO	9.13± ^{Bh} 0.01	9.25± ^{Ae} 0.03	8.90± ^{Ch} 0.00	8.82±0.01 ^{Di}
0.25%RS + CHO	9.40± ^{Cg} 0.01	9.46± ^{Ad} 0.01	9.45±0.01 ^{Bf}	9.39±0.01 ^{Df}
0.5%RS + CHO	9.44± ^{Cf} 0.00	9.48± ^{Ad} 0.01	9.46±0.01 ^{Be}	9.27±0.01 ^{Dg}
0.75%RS + CHO	9.48±0.00 ^{Ce}	9.56± ^{Ad} 0.02	9.53±0.02 ^{Bd}	9.46± ^{De} 0.01

* Small shared letters indicate no significant differences in each column and large shared letters indicate no significant difference in each row

3.2 Acidity and Ph Values in Cheeses with Different Treatments

Full-fat and blank CHO treatments had the highest and the lowest acidity respectively during cheese ripening (Table 4). Acidity of cheeses made with both of the EPS⁺ and the different concentrations of resistant starch were significantly higher than the acidity of cheeses made without the EPS⁺ and with the different concentrations of resistant starch ($P<0.05$).

It likely seems the EPS may have a protective effect on the viability of LAB strains which have resulted in a greater acidity [32]. Although, by increasing the ripening period, acidity increased in all treatments; the highest increasing acidity observed in the third week. Also, the highest count of LAB strains was seen at this time. After this week a slight increasing was observed in the acidity of all treatments. Probably, with progress of cheese ripening period, it was observed that amount of lactose was decreased. Also the inhibitory effect of lactic acid on some strains of LAB caused to reduce the producing acidity amount [33].

Full-fat and blank CHO treatments had the lowest and the highest pH value respectively during cheese ripening (Table 5). In addition, in the third and sixth weeks, pH value of cheese samples made with both EPS⁺ and different concentrations of resistant starch was significantly lower than pH value of cheese samples made without EPS⁺ and with different concentrations of resistant starch ($P<0.05$). The pH of all treatments was decreased from the first to the third week and then was increased significantly up to the eighth week ($P<0.05$). It is likely that from the first to the third week, the conversion of lactose to lactic acid by the selected cultures of lactic acid bacteria (either CHOOZIT or ST- body2+rR704) decrease the pH value. Cheese proteolysis during ripening caused a release of free amino acids that increased the pH value to a somewhat higher level [34].

Table 4. Acidity of cheeses with different treatments during ripening time (mean \pm standard deviations)

Time treatments	First week	Third week	Sixth week	Eighth week
Full fat	1.75 \pm 0.04 ^{Ca*}	1.96 \pm 0.04 ^{Ba}	2.00 \pm 0.00 ^{Ba}	2.12 \pm 0.03 ^{Aa}
Low fat	1.54 \pm 0.05 ^{Bbcd}	1.93 \pm 0.01 ^{Aa}	1.97 \pm 0.02 ^{Ab}	1.99 \pm 0.04 ^{Bb}
Blank with EPS	1.62 \pm 0.01 ^{Cb}	1.79 \pm 0.01 ^{Bd}	1.84 \pm 0.01 ^{Bde}	1.91 \pm 0.00 ^{Ad}
0.25%RS + EPS	1.59 \pm 0.04 ^{Cbc}	1.88 \pm 0.01 ^{Bb}	1.90 \pm 0.01 ^{ABc}	1.94 \pm 0.01 ^{Ad}
0.5%RS + EPS	1.55 \pm 0.05 ^{Cbc}	1.85 \pm 0.02 ^{Bc}	1.87 \pm 0.04 ^{Bc}	1.99 \pm 0.01 ^{Abc}
0.75%RS + EPS	1.60 \pm 0.05 ^{Cb}	1.89 \pm 0.00 ^{Bb}	1.84 \pm 0.02 ^{ABb}	2.00 \pm 0.06 ^{Ab}
Blank CHO	1.36 \pm 0.03 ^{Df}	1.72 \pm 0.01 ^{Ce}	1.78 \pm 0.1 ^{Bi}	1.85 \pm 0.25 ^{Ae}
0.25%RS + CHO	1.46 \pm 0.04 ^{Ude}	1.76 \pm 0.02 ^{Cd}	1.83 \pm 0.00 ^{Be}	1.90 \pm 0.24 ^{Ade}
0.5%RS + CHO	1.42 \pm 0.01 ^{Def}	1.77 \pm 0.00 ^{Cd}	1.85 \pm 0.01 ^{Bde}	1.92 \pm 0.01 ^{Ad}
0.75%RS + CHO	1.51 \pm 0.07 ^{Ccd}	1.78 \pm 0.01 ^{Bd}	1.90 \pm 0.00 ^{Ac}	1.95 \pm 0.01 ^{Ac}

* Small shared letters indicate no significant differences in each column and large shared letters indicate no significant difference in each row

Table 5. pH in cheeses with different treatments during storage (mean \pm standard deviation)

Time treatments	First week	Third week	Sixth week	Eighth week
Full fat	4.55 \pm 0.00 ^{ABc*}	4.38 \pm 0.01 ^{Cd}	4.47 \pm 0.02 ^{Be}	4.57 \pm 0.01 ^{Ae}
Low fat	4.75 \pm 0.02 ^{Abc}	4.48 \pm 0.01 ^{Dc}	4.54 \pm 0.01 ^{Cd}	4.68 \pm 0.02 ^{Bd}
Blank with EPS	4.75 \pm 0.00 ^{Abc}	4.54 \pm 0.00 ^{Ca}	4.66 \pm 0.01 ^{Bab}	4.75 \pm 0.00 ^{Abc}
0.25%RS + EPS	4.71 \pm 0.00 ^{Ad}	4.49 \pm 0.01 ^{Cbc}	4.61 \pm 0.26 ^{Bc}	4.73 \pm 0.00 ^{Ac}
0.5%RS + EPS	4.75 \pm 0.02 ^{Ac}	4.50 \pm 0.00 ^{Cbc}	4.63 \pm 0.02 ^{Bbc}	4.74 \pm 0.02 ^{Ac}
0.75%RS + EPS	4.72 \pm 0.01 ^{Ad}	4.50 \pm 0.00 ^{Cbc}	4.61 \pm 0.02 ^{Bb}	4.74 \pm 0.06 ^{Ac}
Blank CHO	4.78 \pm 0.01 ^{Aa}	4.56 \pm 0.03 ^{Ca}	4.69 \pm 0.01 ^{Ba}	4.78 \pm 0.00 ^{Aa}
0.25%RS + CHO	4.77 \pm 0.00 ^{Aab}	4.55 \pm 0.00 ^{Ca}	4.66 \pm 0.01 ^{Bab}	4.76 \pm 0.00 ^{Aab}
0.5%RS + CHO	4.78 \pm 0.00 ^{Aab}	4.51 \pm 0.01 ^{Db}	4.60 \pm 0.01 ^{Cc}	4.75 \pm 0.00 ^{Bbc}
0.75%RS + CHO	4.77 \pm 0.01 ^{Aab}	4.54 \pm 0.01 ^{Da}	4.65 \pm 0.01 ^{Cb}	4.74 \pm 0.00 ^{Cbcz}

* Small shared letters indicate no significant differences in each column and large shared letters indicate no significant difference in each row

3.3 Moisture and Fat in Cheeses with Different Treatments

Table 6 shows that the full-fat cheese had the lowest moisture content compared with the cheeses made from low-fat retentate during cheese storage. The differences in moisture content between the full-fat cheese and the low-fat cheese treatments may be related to their protein content; a higher protein content of low-fat cheeses contribute to increased

water-binding capacity of the cheese matrix [35]. The moisture content of blank low fat cheese made with the EPS⁺ treatment was significantly higher than the blank low fat cheese without EPS⁺ treatment during cheese storage ($P<0.05$). This finding is in agreement with the results of Costa et al. [36]. In the eighth week, the moisture content of cheese treatments made with both EPS⁺ and different concentrations of resistant starch was significantly higher than the moisture content of cheese treatments made without EPS⁺ and with different concentrations of resistant starch ($P<0.05$). This finding is in agreement with the results of Zisu and Shah [37]. They reported that application of fat replacers and EPS⁺ into cheese milk increase the curd moisture retention during cheese storage. In this study, moisture content of all treatments was decreased from the first to third week and then was increased significantly up to the eighth week ($P<0.05$).

In the third and sixth weeks, the fat content of cheese treatments made with both of the EPS⁺ and the different concentrations of resistant starch was significantly lower than the fat content of cheeses made without the EPS⁺ and with the different concentrations of resistant starch ($P<0.05$) (Table 7). In agreement with this finding, Costa et al. (2012) [36] showed that application of EPS⁺ in low fat and half fat cheddar cheese results resulted in decreasing fat% compared with the treatments without EPS⁺ [36]. In the third week, the fat content of most treatments was decreased. With an increase in the ripening period, a slight decreasing was observed in the fat content of most treatments due to the breakdown of fat into FFA and finally to volatile flavorful compounds [38].

Table 6. Moisture content in cheeses with different treatments during storage (mean \pm standard deviation)

Time treatments	First week	Third week	Sixth week	Eighth week
Full fat	66.04 \pm 0.4 ^{Ae*}	63.32 \pm 0.32 ^{Df}	63.97 \pm 0.07 ^{Cg}	65.68 \pm 0.11 ^{Bf}
Low fat	70.95 \pm 0.31 ^{Ab}	68.70 \pm 0.33 ^{Cd}	70.09 \pm 0.14 ^{Be}	70.57 \pm 0.64 ^{Ac}
Blank with EPS	70.89 \pm 0.11 ^{Ab}	70.44 \pm 0.06 ^{Cb}	70.61 \pm 0.04 ^{Bbc}	70.79 \pm 0.06 ^{Ab}
0.25%RS + EPS	71.71 \pm 0.1 ^{Aa}	70.85 \pm 0.05 ^{Ca}	70.94 \pm 0.09 ^{BCa}	71.09 \pm 0.13 ^{Ba}
0.5%RS + EPS	70.92 \pm 0.31 ^{Ct}	70.39 \pm 0.04 ^{Bcd}	70.73 \pm 0.08 ^{ABe}	70.75 \pm 0.1 ^{Ad}
0.75%RS + EPS	70.74 \pm ^{Cb} 0.14	69.99 \pm ^{Bcd} 0.05	70.39 \pm 0.12 ^{Ad}	70.66 \pm ^{Ad} 0.03
BlankCHO	69.2 \pm ^{Cd} 0.20	68.55 \pm ^{De} 0.62	69.57 \pm ^{Bf} 0.21	70.05 \pm 0.06 ^{Ae}
0.25%RS + CHO	71.44 \pm ^{Ab} 0.07	70.00 \pm ^{Bc} 0.14	70.1 \pm 0.11 ^{Be}	70.55 \pm 0.02 ^{Ad}
0.5%RS + CHO	70.43 \pm 0.09 ^{Ac}	70.00 \pm ^{Cc} 0.05	70.22 \pm 0.02 ^{Bde}	70.39 \pm 0.06 ^{Ad}
0.75%RS + CHO	70.81 \pm 0.3 ^{Ab}	70.71 \pm ^{Bbc} 0.04	70.34 \pm 0.27 ^{Bde}	70.58 \pm ^{ABe} 0.16

* Small shared letters indicate no significant differences in each column and large shared letters indicate no significant difference in each row

3.4 Sensory Evaluation in Cheeses with Different Treatments

In hedonic test, all the sensory characteristics of full fat treatment was the most preferred. However, in all sensory attributes except spread ability, there is no significant different between full fat and 0.75% RS+EPS treatments. Also, except spread ability and overall acceptability, 0.25% RS+EPS and full fat treatments were similar in terms of volunteer compliance. Most sensory characteristics of cheese treatments made with both EPS⁺ and different concentrations of resistant starch had significantly more preference than the cheese treatments made without EPS⁺ and with different concentrations of resistant starch ($P<0.05$) (Fig. 2).

Table 7. Fat content in cheeses with different treatments during storage (mean ± standard deviation)

Time treatments	First week	Third week	Sixth week	Eighth week
Full fat	15.66± 0.29 ^{Aa*}	16.00±0.01 ^{ABa}	15.33±0.29 ^{Ba}	15.33±0.29 ^{Ba}
Low fat	7.50±0.00 ^{Ab}	7.60±0.29 ^{Ab}	7.33±0.29 ^{Ab}	6.83±0.29 ^{Bb}
Blank with EPS	7.50±0.00 ^{Ab}	6.50±0.00 ^{Bd}	6.50±0.00 ^{Bc}	6.83±0.29 ^{Bbc}
0.25%RS + EPS	7.29±0.00 ^{Ab}	6.50±0.00 ^{Bd}	6.50±0.00 ^{Bc}	6.17±0.29 ^{Cc}
0.5%RS + EPS	7.50±0.00 ^{Ab}	6.50±0.00 ^{Bd}	6.50±0.00 ^{Bc}	6.33±0.29 ^{Bbc}
0.75%RS + EPS	7.66± ^{Ab} 0.29	6.66± ^{Bd} 0.29	6.50±0.00 ^{Bc}	6.50± ^{Bbc} 0.06
Blank CHO	7.66± ^{Ab} 0.29	7.16± ^{Bc} 0.29	7.16± ^{Bb} 0.29	6.50±0.00 ^{Cbc}
0.25%RS + CHO	7.50± ^{Ab} 0.29	7.16± ^{Bc} 0.29	7.16± ^{Bb} 0.29	6.50±0.00 ^{Cb}
0.5%RS + CHO	7.66±0.29 ^{Ab}	7.33± ^{ABbd} 0.29	7.00±0.00 ^{Bb}	6.50±0.00 ^{Cb}
0.75%RS + CHO	7.50±0.00 ^{Ab}	7.16±0.29 ^{Ac}	7.16±0.29 ^{Ab}	6.83± ^{Ab} 0.76

* Small shared letters indicate no significant differences in each column and large shared letters indicate no significant difference in each row

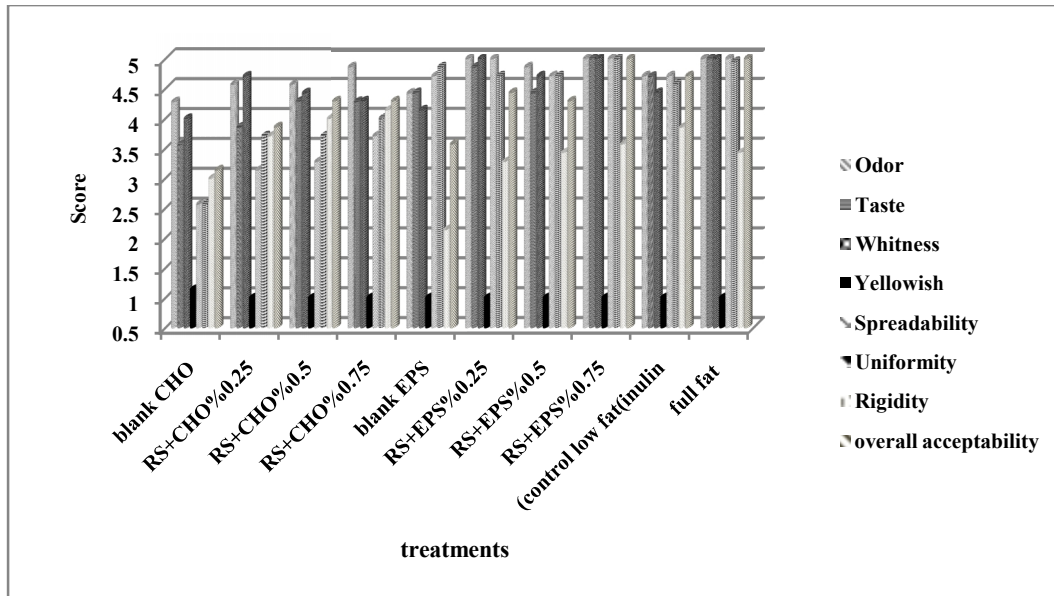


Fig. 2. Effect of different treatments of RS and EPS on the sensory properties at eighth week of storage at 5°C

4. CONCLUSION

The present work is clearly reporting that the viability of the starter culture in low fat UF Feta cheese can be improved by addition of both resistant starch and exopolysaccharide-producing starter culture over 60 days of ripening period. The results indicated that acidity of cheeses made with both the exopolysaccharide-producing starter and the different concentrations of resistant starch was higher than the acidity of cheeses made without the EPS⁺ and the resistant starch ($P<0.05$). Also, pH value of cheese samples made with both EPS⁺ and different concentrations of resistant starch was significantly lower than cheese samples made without EPS⁺ and with different concentrations of resistant starch. These

results were confirmed by bacterial enumeration and revealed that cheeses made with the combination of EPS and 0.25% resistant starch had a positive effect on viable counts of bacteria. Besides, this combination had the highest moisture content compared with other samples. The sensory evaluation of cheese treatments demonstrated that 0.75% RS+EPS received the highest panelists' scores.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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