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Effects of *Bifidobacterium bifidum* and *Enterococcus faecium* incorporation on qualitative attributes of Iranian ultra-filtrated Feta cheese

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Abstract

The present work determined the effect of *Bifidobacterium bifidum* and *Enterococcus faecium* incorporation on the qualitative attributes of ultra-filtrated (UF) Feta cheese. The alterations in pH, titratable acidity, proteolysis, and lipolysis were evaluated during 60 days of refrigerated storage. Viable count, optical analysis, and sensory evaluation were also performed on the freshly made cheeses throughout the storage period. Results showed that incorporating *B. bifidum* and *E. faecium*, either individually or in combination, significantly decreased the pH values as compared to control. Cheeses incorporated with *E. faecium* had significantly higher titratable acidity, proteolysis, and lipolysis than the other treatments. Probiotics counts were higher than 10^6 CFU/g at the end of the storage period. Co-inoculation of both probiotics did not enhance the viability of either. Moreover, the colour of UF Feta cheeses was not influenced by the incorporation of *B. bifidum* and *E. faecium*. The other sensory features remained unchanged on the first day of refrigerated storage. Overall, *B. bifidum* and *E. faecium* could be promising species for industrial production of probiotic UF Feta cheeses.

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Introduction

Probiotics are defined as 'live microorganisms which confer health benefits to the host when administered in adequate amounts' (Hill *et al.*, 2014). Most commercially available probiotic microorganisms comprise the strains of *Lactobacillus* and *Bifidobacterium* genera. The strains of *Bacillus*, *Pediococcus*, *Enterococcus*, and some yeasts have also shown potential to be applied as probiotics (Soccol *et al.*, 2010; Hassanzadazar *et al.*, 2014). The value of global probiotic commerce in 2013 was $32.06 billion, and it was predicted to possibly develop to $73.8 billion in 2024 (Zucko *et al.*, 2020). They may improve the human digestive tract through some mechanisms including inhibiting pathogenic growth, competing with the pathogens, obstructing pathogenic adhesion sites, regulating immune system, and interrupting toxin receptors. Probiotics may also exert protective effect on epithelial cells against gliadin-derived allergens (Vanderpool *et al.*, 2008; Orlando *et al.*, 2018; Norouzbeigi *et al.*, 2020b). The incorporation of probiotics and prebiotics into food products can effectively reduce the occurrence of chronic diseases (Shafi *et al.*, 2019). To receive the benefits of probiotic foods, the foods should possess viable probiotics in adequate amounts within their shelf life (Massoud *et al.*, 2014). Dairy products are considered excellent carriers that can preserve sufficient probiotic populations (10^6 – 10^7 CFU/mL or g) to represent functional properties in hosts (Ranadheera *et al.*, 2018). Carriers can influence probiotics' beneficial characteristics such as resistance in the gastrointestinal tract, sticking to the intestinal epithelium, modulation of the immune system, and antimutagenic, as well as antagonistic features (Norouzbeigi *et al.*, 2020a). The applied probiotic strains and the incorporated milk products

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can influence the health benefits of probiotics (Grom *et al.*, 2020).

In comparison with high acidic fermented milk products, cheese contains special physicochemical features such as (a) higher pH value, (b) lower titratable acidity, (c) higher buffering capacity, (d) higher fat contents, (e) higher nutrients, (f) lower oxygen, and (g) denser matrix which are considered desirable characters for delivering probiotics to the hosts (Karimi *et al.*, 2012b; Homayouni *et al.*, 2020). Furthermore, probiotics can enhance the storage and safety of cheeses by preventing spoilage and pathogenic bacterial growth (Rolim *et al.*, 2020). For instance, *Lb. rhamnosus* GG in Minas Frescal cheeses had an inhibitory effect on *Listeria monocytogenes* (Prezzi *et al.*, 2020). Ultra-filtrated (UF)-Feta cheese from pasteurised UF milk is highly desirable in Iran. Iranian UF Feta cheese is categorised as semi-hard cheese by applying rennet and starter media during cheese production. To the best of our knowledge, information on the enrichment of UF Feta cheeses with probiotics is highly scarce. Therefore, the present work aimed to determine the effect of *E. faecium* and *B. bifidum* on the qualitative attributes of Iranian UF Feta cheeses.

Materials and methods

Probiotics, starter culture, and cheese production materials

E. faecium ATCC 6057 and *B. bifidum* BB-12 were obtained from Zist Takhmir Co. (Tehran, Iran). The starter culture consisting of mesophilic-thermophilic bacteria (a combination of *Lactococcus cremoris*, *Lactobacillus lactic*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Streptococcus thermophilus*) was purchased from Chr. Hansen Co. (Harsholm, Denmark). Rennet was purchased from Ceska-lase Co. (Nieuwegein, the Netherlands). Fresh cow milk, apparatus, and filtration module were contributed by Pegah Co. (Tehran, Iran).

Cheese production

UF Feta cheese was produced according to Robinson and Tamime (1996). Briefly, fat and skim milk were recombined up to a standard fat content (3.5%) after the fat had been separated by microfiltration. Afterward, the milk was pasteurised (72°C/15 s). The UF was operated on the pasteurised milk in three successive loops (loop one, two, and three constituted twelve, nine, and six filters, which concentrated milk Brix up to 16°, 21°, and 28° at each step, respectively). The volume concentration factor of the UF process was 2:9 (kg retentate:kg inlet milk). The homogenisation was carried out under 7×10^3 kPa/55°C/2 s condition. Then, the homogenised retentate was pasteurised at 78°C/15 s. Immediately, it was cooled down to 32°C. In the next step, 2% w/v starter culture and 5% rennet were consecutively added to the retentate. Then, polystyrene containers (thickness: 0.45 mm) were filled with 450 g of the retentate. The initial counts of *B. bifidum* and *E. faecium* were both ~12 log CFU/g in the freeze-dried powders of probiotics, and 1 g was added to each litre of milk. Four treatments namely C-UF (UF Feta cheese without any probiotic; served as control), B-UF (UF Feta cheese containing *B. bifidum* at a final concentration of 10^8 CFU/g), E-UF (UF Feta cheese containing *E. faecium* at a final concentration of 10^8 CFU/g), and BE-UF (UF Feta cheese containing 10^8 CFU/g *B. bifidum* and 10^8 CFU/g *E. faecium*) were prepared. The probiotics were added to the containers, and the containers were then moved into a coagulation tunnel where the conversion of retentate to a pre-cheese blend took place (37°C/20 min). In a sealer (Primodan), 2% granular NaCl was deposited on the parchment paper above the pre-cheese blend. The UF Feta cheese containers were sealed using aluminium foil (thickness: 40 µm). In the pre-ripening step, the cheeses were incubated at 30 ± 1/24 h. Finally, the samples were placed in a commercial refrigerator, and they remained there until the end of refrigerated storage (60 days at 4°C). Sampling was performed at certain intervals during refrigerated storage to evaluate UF Feta cheeses qualitative parameters.

Chemical analyses

pH and titratable acidity

A Metrohm pH meter (Metrohm Co., Switzerland) was used to determine the pH during the storage period. Prior to measuring the pH, cheese slurry was obtained by mixing 20 g of grated cheese with 12 mL of distilled water. The titratable acidity (TA) was measured after blending 20 g of Feta cheese sample with 250 mL of purified water, and then filtration through Whatman filter paper number F2042 grade. Finally, 25 mL of filtrate was titrated by 0.1 N NaOH using phenolphthalein (Merck, Germany) as an indicator (Karimi *et al.*, 2012a).

Proteolysis

The total nitrogen (TN) was determined using the macro-Kjeldahl procedure. Water-soluble nitrogen (WSN) and non-protein nitrogen (NPN) were measured to determine the primary and secondary proteolysis.

WSN measurement

Briefly, 30 g of Feta cheese sample was homogenised with 60 mL of distilled water in a stomacher (Stomacher® 400 Circulator, Seward, UK) for 5 min. Afterward, the pH of the mixture was adjusted to 4.6 using 2 N HCl (Merck, Germany) and 2 N NaOH (Merck, Germany). After 30 min, the pH was readjusted to 4.6, then the mixture was incubated at 40°C for 30 min. The mixture was centrifuged to separate the insoluble solids (4°C/30 min/ 4,000 *g*). Finally, the supernatant was filtered using Whatman paper no. 1 and glass wool. The WSN was determined using the macro-Kjeldahl method (Kuchroo and Fox, 1982).

NPN measurement

The filtrate was obtained according to the WSN procedure. Then, 20 mL of the filtrate was mixed with 5 mL of trichloroacetic acid (60% solution; Merck, Germany), and was incubated at ambient temperature for 30 min. It was then centrifuged at 5,000 *g* for 10 min, and the supernatant was filtered using Whatman paper no. 1. The NPN was also assessed by the macro-Kjeldahl method (Kuchroo and Fox, 1982).

Lipolysis

The acid degree value (ADV) was measured through titration. In short, 6 g of anhydrous Na_2SO_4 (Sigma Aldrich, Germany) was added to 10 g of grated Feta cheese, and blended with 60 mL of diethyl ether (Merck, Germany). The mixture was incubated for about 1 h at 25°C and homogenised. Afterward, centrifugation was done on the mixture (4,500 *g*/10 min/4°C), and the supernatant was filtered using Whatman paper no. 1. Lastly, the supernatant was titrated using 0.1 N KOH (Nunez *et al.,* 1986). For chemical analyses, Feta cheese samples were gathered at 30-day intervals during 60 days of refrigerated storage.

Optical analysis

The colour parameters including *L**, lightness; *a**, greenness to redness; and *b**, blueness to yellowness were measured using a HunterLab colorimeter model ColorFlex EZ (Virginia, USA) according to CIELAB scales (Mazinani *et al.,* 2016). The colour parameters were assessed on the first day of refrigerated storage.

Microbiological analysis

Briefly, 25 g of grated UF Feta cheese sample was transferred into an Erlenmeyer flask containing 225 mL of sterile water. Then, 2% (w/v) trisodium citrate (Sigma Aldrich, Germany) was added to the mixture at 40°C. The mixture was then homogenised in a stomacher (Stomacher® 400 Circulator, Seward, UK) for 5 min at high speed to produce a slurry mixture representing the first dilution. The subsequent serial dilutions were applied in sterile water constituting 1% (w/v) peptone water. The probiotics were selectively counted using de Man, Rogosa, and Sharpe (MRS) bile agar (bile was purchased from Sigma Aldrich, Germany; MRS was purchased from Merck, Germany). The inoculated plates were incubated at 37°C for 72 h (Tharmaraj and Shah, 2003). The microbiological evaluation was performed at 15-day intervals during 60 days of refrigerated storage.

Sensory analysis

A sensory panel consisting of 25 semi-trained men and women (10 men and 15 women), aged 20 to 45, from students and staff of Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran participated in this analysis. A hedonic 5-point scale, in which '5' indicated 'most like' and '1' indicated 'most dislike', was used to assess the sensory parameters including colour, oral texture, and flavour (Norouzbeigi *et al.,* 2020a).

Statistical analysis

Each UF Feta cheese treatment was produced thrice, and the determinations were conducted in triplicate. SPSS (V26) was used to analyse the collected data with a confidence level of 95% to detect any significance among cheese treatments. The microbiological analysis was assessed using repeated measure ANOVA. The chemical analyses were assessed using One-way ANOVA. In both cases, the Bonferroni *post-hoc* experiment was employed for comparing the means. Non-parametric tests were applied to analyse the sensorial properties.

Results and discussion

Changes in pH and acidity during storage period

Figure 1 shows that pH decreased while titratable acidity increased throughout refrigerated storage. Five individualised phases, including lag, pre-log, log, late log, and stationary phases were observed. pH and TA are considered principal factors for measuring the acidity of probiotic-fermented dairy products. The breakdown of lactose to lactic acid throughout incubation leads to specific pH, coagulation, and gel formation. The bacterial strains that participate in the incubation mainly influence fast or slow pH drop rates (Yerlikaya *et al.*, 2020). The presence of *B. bifidum* and *E. faecium* in cheese treatments individually or in combination resulted in significantly lower pH values than the control ($p < 0.05$). The pH values of 4.77, 4.76, 4.75, and 4.76 on the first day of storage (d0) in control, B-UF, E-UF, and BE-UF decreased to 4.54, 4.51, 4.49, and 4.48 on the last day of storage (d60), respectively. Significant differences in pH values were not observed between probiotic treatments. The amounts of TA from 0.81, 0.81, 0.82, and 0.81 at d0 in control, B-UF, E-UF, and BE-UF increased to 0.89, 0.90, 0.94, and 0.95, respectively at d60. The TA values in E-UF and BE-UF were significantly greater than control and BE-UF ($p < 0.05$).

Similarly, Kwark cheese supplemented with *B. longum* KACC 91563 had a lower pH than control treatments (Song *et al.*, 2017). In an investigation performed by Magdoub *et al.* (2005), a decrease in pH in Ain Shams cheese was observed, and might be attributed to the conversion of the remaining lactose to the lactic and free fatty acid. In another research, cheeses made with *E. feacalis* and *E. feacium* had a lower pH than control. As a result of the starter bacterial and probiotics' growth and acid formation, the pH values decreased quickly during ripening. A dramatic decrease in pH values at the beginning of cheese manufacturing is considered essential in cheese-making because it is important for coagulation and growth inhibition of opportunist microbiota (Sarantinopoulos *et al.*, 2001; Rasouli Pirouzian *et al.*, 2012).

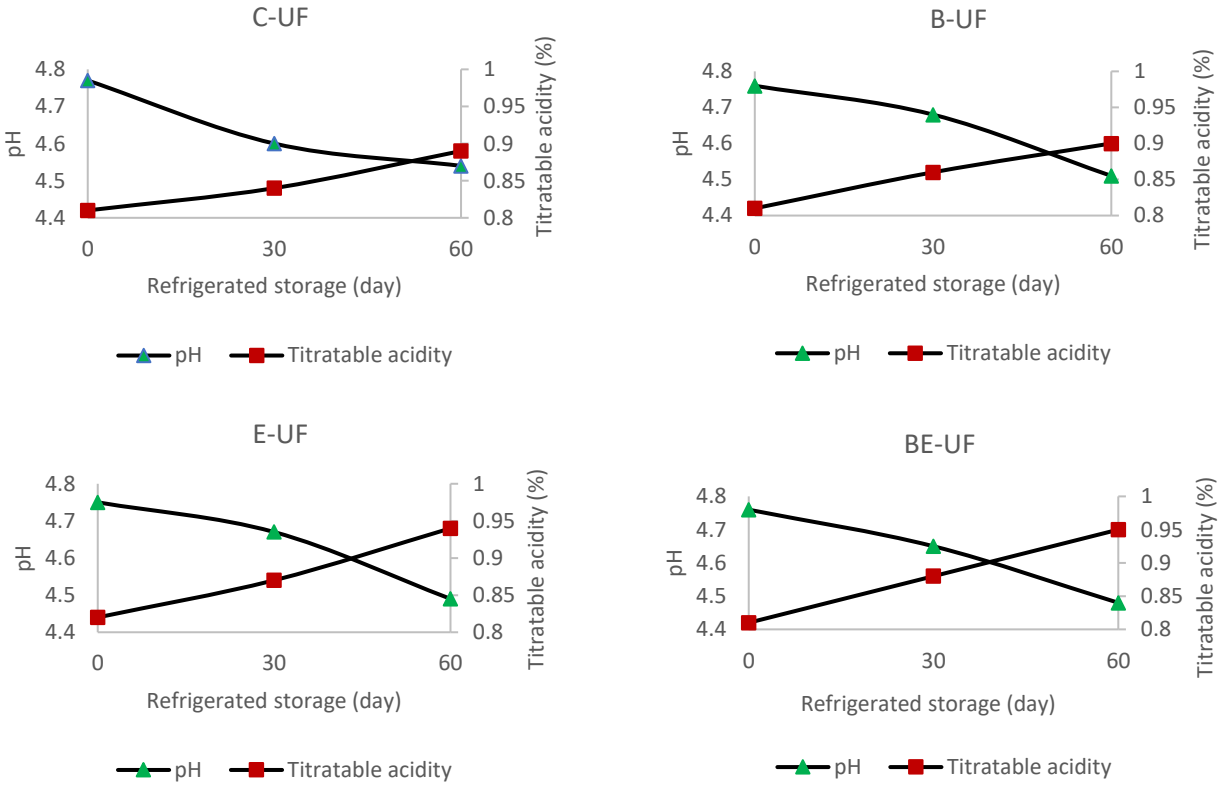

Figure 1. Changes in pH and titratable acidity during refrigerated storage (5°C, 60 days). Treatments: C-UF = control Feta cheese ; B-UF = Feta cheese containing *B. bifidum*; E-UF = Feta cheese containing *E. faecium*; and BE-UF = Feta cheese containing *B. bifidum* and *E. faecium*.

Viability of probiotic

Figure 2 shows the probiotics' viability during 60 days of refrigerated storage in UF Feta cheeses. The decreasing trends of probiotic's viability of all treatments were apparent. The probiotic counts in treatments containing *B. bifidum* and *E. faecium* significantly decreased from 7.65 and 7.94 log CFU/g at day 0 to 6.46 and 6.62 log CFU/g at day 60, respectively ($p < 0.05$). A significant decrease in *B. bifidum* viability occurred in B-UF during the first 15 days of storage by about 85%. A slight decrease by about 53% (from 6.85 to 6.46 log CFU/g) was observed throughout the refrigerated storage. On the contrary, E-UF showed a gradual decrease in the viability of *E. faecium* during 60 days of rerigerated storage. The highest decrease in the probiotic count took place during the first month of refrigerated storage by about 90% (from 7.92 to 6.90 log CFU/g).

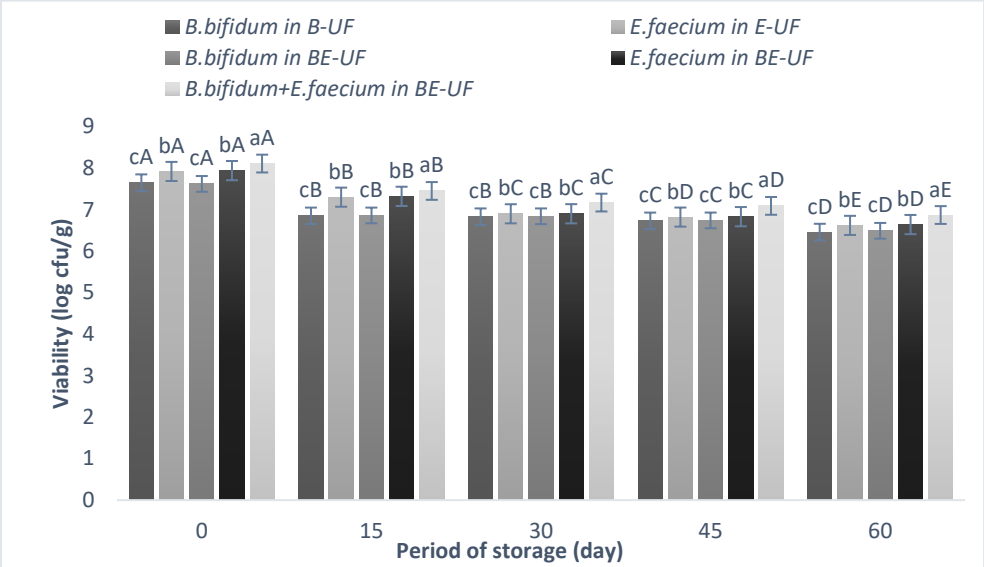

Figure 2. Changes in viability during refrigerated storage (5°C, 60 days). Means in the same day, but on a different treatment, shown with different lowercase letters are significantly different ($p < 0.05$). Means in the same treatment, but on a different day, shown with uppercase letters are significantly different ($p < 0.05$). Treatments: C-UF = control Feta cheese ; B-UF = Feta cheese containing *B. bifidum*; E-UF = Feta cheese containing *E. faecium*; and BE-UF = Feta cheese containing *B. bifidum* and *E. faecium*.

By comparing B-UF and E-UF, it can be concluded that *B. bifidum* was more sensitive to environmental conditions in Feta cheese than *E. faecium*, especially during the first 15 days of refrigerated storage. Additionally, the probiotic count in B-UF decreased to below 7 log CFU/g after 15 days. Considering 10^6 CFU/g as the minimum count to consider cheese a probiotic product, the probiotics' concentrations in all the treatments were higher than this cut-off point even after 60 days (Ranadheera *et al.*, 2018).

NaCl may possess inhibitory effects against probiotics (Gomes *et al.*, 1998). Gobbetti *et al.* (1998) reported that NaCl provided considerable inhibition against probiotic strains when the salt concentration exceeded 4% (wt/wt) of cheese. In the present work, we added 2% (wt/wt) NaCl to the UF Feta cheese formulation which could mildly interfere with probiotic bacteria's survival during the storage period. NaCl could inhibit probiotic growth due to osmotic effect (Guinee, 2004). Another factor affecting the probiotic viability during shelf life is the oxygen permeation rate from the cheese packaging (Norouzbeigi *et al.*, 2020a). Polystyrene has a relatively high permeability to oxygen molecules which is fatal for probiotics (Norouzbeigi *et al.*, 2020a; Polymer Properties Database, 2021).

Regarding the treatment having both probiotic strains, neither synergistic nor antagonistic effect between *E. faecium* and *B. bifidum* was observed. The co-inoculation of both probiotics did not lead to enhancing the viability of either.

Proteolysis activity during refrigerated storage

Proteolysis is considered essential for cheese manufacturing, and includes several biochemical reactions occurring during ripening. Proteolysis is the hydrolysis of casein to peptides and amino acids by several proteinases and peptidases from some origins such as (a) milk, (b) coagulant, (c) starter bacteria, (d)

non-starter bacteria, (e) ripening cultures, and (f) proteolytic enzymes (Ardö *et al.,* 2017). Proteolysis influences not only cheese's texture by hydrolysing protein matrices, but also affects cheese's taste by creating lightweight molecular components (Ivanov and Markova, 2020). UF white cheese is specified by lower proteolysis and amino acids' generation within the ripening period (Hesari *et al.*, 2006). As shown in Figure 3, the NPN and WSN values, that are indicators of the amount of proteolysis in E-UF and BE-UF, were significantly higher than control and B-UF from the middle to the end of refrigerated storage ($p < 0.05$).

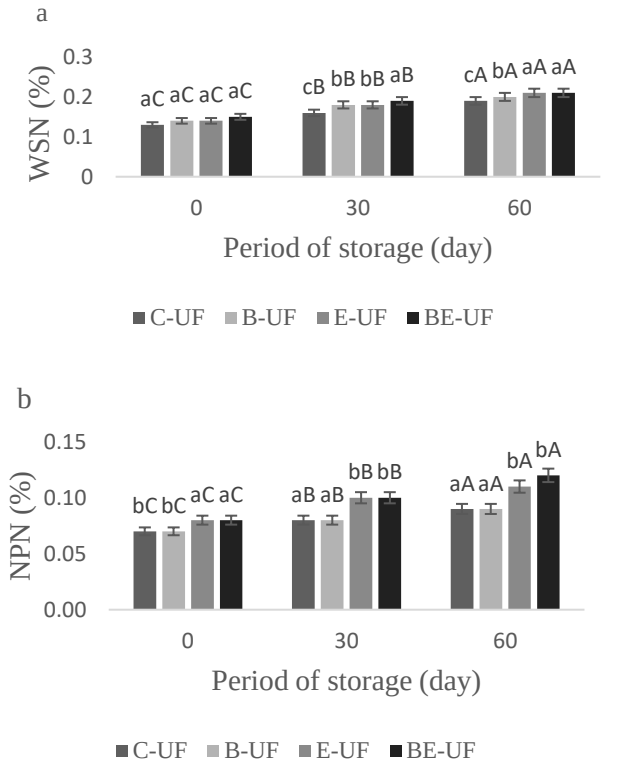

Figure 3. Changes in proteolytic activity, **(a)** WSN and **(b)** NPN, during refrigerated storage (5°C, 60 days). Means in the same day, but on a different treatment, shown with different lowercase letters are significantly different ($p < 0.05$). Means in the same treatment, but on a different day, shown with uppercase letters are significantly different ($p < 0.05$). Treatments: C-UF = control Feta cheese ; B-UF = Feta cheese containing *B. bifidum*; E-UF = Feta cheese containing *E. faecium*; and BE-UF = Feta cheese containing *B. bifidum* and *E. faecium*.

The NPN values of 0.07, 0.07, 0.08, and 0.08 on the first day of refrigerated storage in control, B-UF, E-UF, and BE-UF increased to 0.09, 0.09, 0.11, and 0.12 at the end of storage, respectively. Also, the WSN values of 0.07, 0.07, 0.08, and 0.08 at d0 in control, B-UF, E-UF, and BE-UF increased to 0.19, 0.20, 0.21, and 0.21 at d60, respectively. Therefore, it seemed that the proteolytic activity or breakdown of casein to peptides and amino acids by *E. faecium* was remarkably greater than *B. bifidum*. In other works, *Bifidobacterium* strains did not demonstrate any proteolytic activity, and thus did not affect the proteolytic pattern of the probiotic cheeses (Dinakar and Mistry, 1994; Corbo *et al.,* 2001; Ong *et al.,* 2006). In another work performed on enterococci's effect on the quality of UF white cheese, treatment containing *E. faecium* exhibited the highest percentage of NPN (Rasouli Pirouzian *et al.,* 2012).

Lipolysis activity during refrigerated storage

Lipolysis is known as an essential biochemical reaction occurring in cheeses as a result of esterase and lipase activity during ripening (Thierry *et al.,* 2017; García-Cano *et al.,* 2020). Based on Table 1, the amounts of lipolysis increased progressively throughout the refrigerated storage.

Table 1. Lipolysis amounts in different treatments during refrigerated storage (5°C, 60 days).

Treatment	Lipolysis (meq KOH/100 g fat)		
	0	**30**	**60**
C-UF	0.11^{bC}	0.68^{bB}	1.55^{bA}
B-UF	0.12^{bC}	0.68^{bB}	1.57^{bA}
E-UF	0.15^{aC}	0.81^{aB}	1.73^{aA}
BE-UF	0.15^{aC}	0.8^{aB}	1.78^{aA}

Means in the same column shown with different lowercase superscripts are significantly different ($p < 0.05$). Means in the same row shown with different uppercase superscripts are significantly different ($p < 0.05$). Treatments: C-UF = control Feta cheese ; B-UF = Feta cheese containing *B. bifidum*; E-UF = Feta cheese containing *E. faecium*; and BE-UF = Feta cheese containing *B. bifidum* and *E. faecium*.

The lipolysis index from 0.11, 0.12, 0.15, and 0.15 on the first day of refrigeration in control, B-UF, E-UF, and BE-UF increased to 1.55, 1.57, 1.73, and 1.78 on the last day of refrigeration, respectively. Treatments containing *E. faecium* showed significantly higher lipolytic activity than other treatments ($p < 0.05$). Differences between control and the treatment containing *B. bifidum* only were not significant ($p > 0.05$). This might be attributed to the

higher lipolytic activity of *E. faecium* when compared with *B. bifidum*. Similarly, in a study that was performed to determine the influence of enterococci on the quality of UF cheese, the incorporation of *E. faecium* and *E. faecalis* led to a greater level of lipolysis index by the end of ripening in comparison to control cheese (Rasouli Pirouzian *et al.*, 2012).

Optical analysis

Colour is a fundamental physical property of food that correlates to food's physicochemical and sensory characteristics. Colour has a pivotal function in appraising the quality of foods, and it commonly influences consumers' choice at first glance (Almutairi, 2016). As illustrated in Table 2, in control, B-UF, E-UF, and BE-UF treatments, respectively, the *L** values were 61.3, 61.4, 61.5, and 61.2, the *a** values were 3.4, 3.7, 3.6, and 3.4, and the *b** values were 3.8, 3.6, 3.6, and 3.9. Significant differences were not detected in *L**, *a**, and *b** values among different treatments on the initial storage date ($p > 0.05$).

Table 2. Colour analysis in the first day of storage.

Treatment	Parameter		
	L*	***a****	***b****
C-UF	61.3	3.4	3.8
B-UF	61.4	3.7	3.6
E-UF	61.5	3.6	3.6
BE-UF	61.2	3.4	3.9

Treatments: C-UF = control Feta cheese ; B-UF = Feta cheese containing *B. bifidum*; E-UF = Feta cheese containing *E. faecium*; and BE-UF = Feta cheese containing *B. bifidum* and *E. faecium*. *L** = lightness; *b** = yellowness-blueness; and *a** = redness-greenness.

Likewise, the incorporation of probiotics could not remarkably change the colour of Kwark cheese in comparison to the control sample (Song *et al.*, 2017). In other work, the incorporation of various probiotic strains did not significantly influence the colour parameters of Himalayan cheese (*Kalari*) at day 1, even though the values of the different colour parameters changed throughout the 30-day storage at 4°C (Mushtaq *et al.*, 2016).

Sensory analysis

In a comparative experiment regarding the sensory properties of cheese samples, there were no significant differences between all treatments in terms flavour, mouthfeel, and colour's perspective ($p > 0.05$), and they obtained similar scores. This indicated that the incorporation of each probiotic, either *B. bifidum* or *E. faecium*, did not influence the UF Feta cheese's sensory characteristics on the first day of storage. In agreement with our results, incorporating *L. casei*, *L. plantarum*, and *B. bifidum* either in microencapsulated or in free form did not influence the sensory properties, including flavour and texture of Iranian UF cheese (Zomorodi *et al.*, 2011). Moreover, in another study, Iranian white cheese containing *B. animalis* and *L. rhamnosus* did not significantly differ from control treatment in terms of sensory characteristics (Mahmoudi *et al.*, 2012).

Evaluating cheeses is considered a complicated process, both from sensory and non-sensory perspectives (Judacewski *et al.*, 2019). Demographic variables (including age, sex, education, and employment) and behavioural variables can affect expectations and perceptions (Cais-Sokolińska *et al.*, 2021). Among consumer perception's sensory methods, sorting techniques have demonstrated great popularity. Sorting contains a naive implement for the sifting of numerous samples from the dataset. Sorting may be helpful to characterise sensory descriptors associated with a specific area where the artisan cheese is produced and may be used in the verification and enrolment procedures of geographical indication of origin of cheeses (Rodrigues *et al.*, 2020).

Conclusion

UF Feta cheese is one of the most prevalent cheeses in Iran. Therefore, it can be a suitable choice for transferring probiotics and their lucrative effects on people's food chains. In the present work, we investigated the impacts of individually inoculating either *B. bifidum*, *E. faecium*, or in combination on UF Feta cheese's qualitative properties. *E. faecium* was more active than *B. bifidum* in lowering the pH value, increasing the acidity, promoting proteolysis, and lipolysis during 60 days of refrigerated storage. However, the sensory properties of UF Feta cheese were not significantly affected as a result of probiotic inoculation. Similarly, the optical properties of probiotic cheese, either alone or in combination, were not significantly altered as compared to the control treatment. Although more than 90% of *B. bifidum* and *E. faecium* viabilities were lost during the 60 days of

refrigerated storage, the bacterial counts in both probiotic treatments were 6.46 and 6.62 log CFU/g at day 60, respectively. In other words, these cheeses could still be considered probiotic even by the end of their shelf life, provided that 10^6 CFU/g is approved as the lower limit for being a probiotic product. It is worth mentioning that *B. bifidum* was considerably susceptible to the intrinsic conditions of Feta cheese during the first 15 days of the ripening period, in a way that its viability lost about 84% in the latest time. Moreover, the co-administration of both probiotics in cheese did not affect the viability of each. In other words, synergistic or antagonistic interactions were not observed due to the co-inoculation of *E. faecium* and *B. bifidum*. Overall, *E. faecium* and *B. bifidum* are promising species to be used in the industrial production of cheeses, which could provide suitable functional food for probiotic transmission into the human body.

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