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Research Article

Characteristics of Iranian Probiotic UF White Cheese

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Cheese is able to transport live probiotics due to its dry matter, fat, and higher pH than yogurt-like products. According to health organizations' emphasis on promoting the consumption of healthy dairy products and consumers' desire to eat healthier foods, in this study, the feasibility of producing probiotic UF cheese containing probiotic strains of *Bifidobacterium bifidum* sp. IR007-113 and *Enterococcus faecium* sp. IBRC-M 10836 as a single or combined study was investigated. The experimental cheeses used in this study include A: UF cheese as a control sample, B: probiotic UF cheese containing *Bifidobacterium bifidum*, C: probiotic UF cheese containing *Enterococcus faecium*, and D: probiotic UF cheese containing a combination of *Bifidobacterium bifidum* and *Enterococcus faecium* strains. Viability of probiotic organisms on the basis of log cfu/g and aroma compounds including (acetaldehyde, diacetyl, acetone, and acetic acid all in micrograms per gram) was assessed during the 60-day ripening period. The results showed that the probiotic bacterial population decreased during the 60-day storage period. However, at the end of the storage period, all experimental cheeses contained probiotic strains above 10^6 cfu/g. The acetaldehyde compound increased during the 60-day storage period, and diacetyl, acetoin, and acetic acid contents decreased during the 60-day storage period except for the control sample. A total of 12 free fatty acids were identified in Iranian probiotic UF white cheese, which had the highest concentration of palmitic acid among saturated fatty acids and oleic acid among unsaturated fatty acids. The sensory scores of flavor increased during the 60-day storage period. Texture sensory scores of all experimental cheeses decreased during the storage period. In terms of acceptability, all experimental cheeses showed an increasing trend. In general, the results showed that it is possible to produce UF probiotic cheese with minimal adverse effects on quality characteristics and sensory acceptability. Also, treatment D had relatively better characteristics compared to other cheese variants.

1. Introduction

Ultrafiltered milk (UF milk), also known as diafiltered milk, is a subclassification of milk protein concentrate that is produced by passing milk under pressure through a thin, porous membrane to separate the components of milk according to its size. Specifically, ultrafiltration allows the smaller lactose, water, mineral, and vitamin molecules to pass through the membrane, while the larger protein and fat molecules (key components for making cheese) are retained and concentrated (depending on the intended use of the UF milk product, the fat in whole milk may be removed before filtration). The removal of water and lactose reduces the volume of milk and thereby lowers its transportation and storage costs. With the significant increase in the production of fermented foods, the interest in using probiotics in dairy products to produce healthy dairy products with nutritional value has increased. Most research has been focused on fermented milks and yogurts, while these products are not optimal for maintaining a high concentration of these strains due to poor survival (especially *Bifidobacterium* strain) in market yogurts. It is an alternative product for the preservation of probiotic strains of cheese because it has more suitable pH, fat, oxygen level, and storage conditions for the

long-term survival of bifidobacteria during processing and digestion. However, since cheese generally possesses a higher pH, and thus, a more stable environment than fermented milk products, probiotic bacteria are more likely to survive over the long term [1]. *Enterococcus* spp. are lactic acid bacteria (LAB) whose natural habitat is the gastrointestinal (GI) tract of humans and animals. They are also found naturally in vegetables, plant material, and other food. They have specific characteristics, such as the ability to survive in moderately harsh conditions and to maintain viability after heating. Because of this tolerance, *Enterococcus* spp. seem to have a potential application in various food systems. *Enterococcus faecium* has good adhesion ability (high values of autoaggregation, coaggregation, and hydrophobicity) [2]. The biopreservative effect can be increased by combining probiotic strains with lactic acid bacteria. Probiotic cheese consumption has been found to reduce exercise-induced immune suppression, treat constipation, and improve body mass index and blood pressure. Many probiotic strains produce antimicrobial compounds, including Bifidobacterium, which reduced Pseudomonas levels in cottage cheese [3]. Cheese flavor development is a complex process in which enzymes—from milk, starter cultures, rennet, and secondary flora—are involved in the degradation of milk proteins, fat, and carbohydrates. Variations in nonstarter lactic acid bacteria (NSLAB) and derived compounds depend on cheese variety and processing and ripening conditions. Starter has an important role during the ripening process, and this may be due to (1) the production of lactic acid, which together with the rennet causes the curd forming, acts as a preservative, and contributes to the acid flavor of cheeses; (2) metabolism of citric acid, which is widely regarded as being essential for flavor production; (3) breakdown of the protein; (4) its contribution to the breakdown of the diglycerides formed from the milk triglycerides by the lipoprotein lipase from the milk; and (5) the breakdown of hippuric acid to benzoic acid. Also, enzymes have an important role during ripening [4]. The characteristic of taste shows the sum of characteristics of the substance that creates that feeling. Flavor and sensory properties are decisive in choosing, accepting, and consuming food. It is obvious that the products from the breakdown of lactose and citric acid (lactic acid, diacetyl, CO_2, etc.), besides those from the breakdown of paracasein (peptides and amino acids), and lipids (free fatty acids) are necessary to create flavor. To create flavor, there must be a correct balance between different flavoring ingredients. Lactic acid produces an acidic flavor that is noticeable in fresh cheese; moreover, although a large amount of lactic acid causes a sour taste in cheese, lactic acid indirectly affects the texture of cheese. A significant change in flavor occurs during cheese ripening. Secondary products resulting from lactose fermentation and partial transformation of lactic acid affect the production of aroma and flavor, including aldehydes, lactones, alcohols, esters, organic acids, and CO_2. Proteolysis plays an important role in creating flavor; paracasein is tasteless; however, peptides may have a bitter taste, and some amino acids have special tastes, especially sweet, bitter, or broth-like tastes. Short-chain peptides and amino acids contribute to the initial flavor of the cheese [4]. UF fresh white cheese prepared by ultrafiltration membrane process is relatively fatty with a soft texture and unique flavor and is prepared from pasteurized cow's milk, starter, rennet, and salt and has a maximum shelf life of 60 days at a temperature of 4–8°C stored in the refrigerator. It is quite obvious that aldehydes, including acetaldehyde, ketone compounds such as acetone and diacetyl, and acids such as acetic acid are the most effective compounds in aroma. The proteolytic activity displayed by some enterococcal strains may contribute to cheese ripening and flavor development. Because of these interesting metabolic traits, enterococci have been proposed as part of a defined starter culture combination for UF white cheeses [5]. Diacetyl is the result of the metabolism of lactose and citrate, which is produced by *lactococci*, and aspartic acid can be mentioned as another source of diacetyl production [6]. Diacetyl was likely produced by the activity of nonstarter lactic acid bacteria in fresh cheese [7]. The flavor of UF Iranian probiotic white cheese is obtained by compounds derived from proteolysis and lipolysis, which are the result of proteolytic and lipolytic activity of starter microorganisms and probiotic bacteria, although severe lipolysis may be considered undesirable in most types of cheese. The triglycerides in all cheese varieties undergo hydrolysis endogenous lipase action, which results in the liberation of fatty acids in cheese; during maturation, the degree of lipolysis in cheese depends on the variety and ranges from slight to very extensive [8]. Fatty acids derived from milk fat positively participate in the flavor of probiotic Iranian UF white cheese and are the precursors of most complex aroma compounds including methyl ketones, alcohols, lactones, and esters. High concentrations of fatty acids cause off-flavor perception in cheese [9]. So, the objective of the present study was to produce Iranian probiotic UF white cheese and to evaluate its characteristics during the storage period.

2. Materials and Method

2.1. Microbial Strains. *E. faecium* and *B. bifidum* were obtained from Zist Takhmir Co. (Tehran, Iran). The starter culture, consisting of mesophilic-thermophilic bacteria (a combination of *Lactococcus cremoris*, *Lactococcus lactic*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Streptococcus thermophilus*), was purchased from Chr. Hansen Co. (Hørsholm, Denmark). Rennet was purchased from Ceskalase Co. (Nieuwegein, the Netherlands). Fresh cow milk, apparatus, and filtration moduli were supplied by Pegah Co. (Tehran, Iran).

2.2. Cheesemaking. Treatments of UF feta cheese were prepared based on the method used by Robinson and Tamime [10]. After the milk fat was separated by a separator, the fat and skimmed milk were mixed until the fat reached 3.5%, and then, the milk was pasteurized at 72°C for 15 s. Ultrafiltration was performed on pasteurized milk in three consecutive rings (rings one, two, and three, which included twelve, nine, and six filters, respectively, and the milk

concentrated to 16 degrees, 21 degrees, and 28 degrees, Brix, respectively, in each stage). The volumetric concentration factor of UF processing was 2 : 9 (kg of retentate: kg of incoming milk), homogenization was conducted under 7×10^3 kPa/55°C/2 s conditions, and then, the homogenized retentate was pasteurized at 78°C for 15 s, and after pasteurization, it was immediately cooled to 32°C. In the next step, 2% w/v of starter culture and 5% of the rennet were added to the retentate, respectively. After this step, the retentate was filled in polystyrene containers (thickness: 0.45 mm) to the amount of 450 grams. The initial count of both *B. bifidum* sp. IR007-113 and *E. faecium* sp. IBRC-M10836 was approximately equivalent to 12 log CFU/g in sachets of lyophilized powders of probiotics, of which one gram was added per liter of milk. Four types of cheese including A, Iranian UF white cheese without any type of probiotics; B, Iranian UF white cheese with probiotics containing *B. bifidum* in a final concentration of 10^8 cfu/mL; C, UF probiotic Iranian white cheese containing *E. faecium* in a final concentration of 10^8 cfu/mL; and D, Iranian UF probiotic white cheese containing 10^8 cfu/mL *B. bifidum* and 10^8 cfu/mL *E. faecium* were prepared in this research. According to the mentioned experimental cheeses, probiotics were added to the containers, and there was also a control sample. The dishes were then transferred to the coagulation tunnel. In this tunnel, the retentate was converted into a precheese mixture (at a temperature of 37°C for 20 min). In a sealer (Primodan), 2% granulated salt was poured on top of the precheese mixture on parchment paper. The containers of UF feta cheese were closed using aluminum foil (thickness: 40 μm). In the preripening step, the cheeses were incubated at $30 \pm 1/24$ h. Finally, the samples were transferred to an industrial refrigerator and kept at a temperature of 4°C until the end of the 60-day storage period. Sampling was carried out at regular intervals during refrigerated storage in order to evaluate the quality characteristics of UF feta cheese.

2.3. Evaluation of the Viability of Probiotic Strains. 2 grams of grated UF feta cheese sample was transferred to 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 homogenized in a Stomacher (Stomacher® 400 Circulator, Seward, UK) for 5 minutes at high speed to prepare a slurry mixture as the first dilution. Subsequent successive dilutions were made in sterile water containing 1% w/v peptone. This dilution was cultured in the RCA (reinforced clostridial agar) medium, which only allowed *Bifidobacterium* to grow, using the pore plate method, and transferred to the incubator under anaerobic conditions (anaerobic jar containing gas pack) at 37°C for 72 h [11]. For the enumeration of *Enterococcus faecium*, an appropriate volume of the selected dilutions was inoculated into the plates containing Bile Esculin Azide Agar and spread using a suitable L-shaped rod, and the plates were placed in an incubator at 37 ± 1°C for 24 h in aerobic conditions [12]. Microbial evaluation was performed at 10-day intervals over 2 months under cold storage conditions.

2.4. Aroma Analysis. First, 4 g of the samples was poured into vials, which were then placed in a hot-water bath. The samples were kept at 50°C for 30 min, during which time the substances responsible for the flavor evaporated and accumulated at the top of the samples. These compounds were analyzed using GC (GC, Varian CP-3800, Amsterdam, Holland). Molecular-weight separation was carried out according to the internal standards prepared from the aroma compounds (acetaldehyde, diacetyl, acetoin, and acetic acid). The concentrations of each compound were reported as the peak areas obtained from GC [13]. To prepare the internal standards, 250 mg of pure aroma compounds (acetaldehyde, diacetyl, acetoin, and acetic acid) was heated using a water bath at 50°C for 30 min. They were then injected into the GC, and the peak areas were calculated. The capillary column was CP-Wax 573 CB (CP 97763) with a 25 m length. The injection was performed using the splitless method, and the flame ionization detector was applied. The experimental conditions for GC were helium gas purity of 99.9%, pressure of 5 psi, column diameter of 0.32 mm, and column thickness of 2.1 μm.

2.5. Free Fatty Acids (FFAs). Extraction of cheese lipids and isolation of the FFA were executed by GC as described by Shahab Lavasani[14]. Samples were prepared as follows: anhydrous Na_2SO_4 (4 g) was used to grind cheese (2.5 g); after this, 0.4 ml H_2SO_4 (2.5 M) and 1.0 ml internal standard solution containing $C_{5:0}$, $C_{7:0}$, $C_{9:0}$, $C_{13:0}$, and $C_{17:0}$ (0.5 mg ml-1each) were added. This mixture was separated three times with 3 ml diethyl ether/heptanes (1 : 1, v/v). The solution was filtered by centrifugation after each extraction (Beckman centrifuge, Model TJ-6, USA) at 2000 rpm for 5 min at room temperature, and the upper solvent layer was transferred to a screw-capped tube containing anhydrous Na_2SO_4 (1.0 g). The pooled diethyl ether/heptane extract was smeared onto a Mega Bond Elut NH2 precolumn (2.8 ml, containing 500 mg of silica adapted with aminopropyl group; Varian, Harbor City, CA, USA), which was prepared with 10 ml heptanes. Using 10 ml chloroform/2-propanol (2 : 1, v/v), the neutral lipids were extracted from the column. The FFA was eluted with 10 ml diethyl ether containing 2% formic acid. A screw-capped tube was used for the collected FFA. A 0.1 μL sample was taken from this solution for GC determination of the FFA. Two chromatographic injections, one from each of the cheese extracts, were produced. Gas chromatography model Star 3400 (Varian, Harbor City, CA, USA) equipped with an on-column injector and a flame ionization detector (FID) was used with capillary column Bp-21 (length 30 m and inner diameter 0.53 mm). Direct cold on-column injection occurred at 60°C for 2 min; the injector temperature increased from 60°C to 220°C at a rate of 10°C min^{-1}, and then, it was maintained at 220°C for 25 min. Injector and detector temperatures were 200°C and 250°C, respectively. Nitrogen at 99.9% purity was used as the carrier gas. Headspace pressure was 15 psig. Identification and quantification of the cheese samples were based on known parameters. Concentrations of different fatty acids were based on a standard (≥99% GC; Sigma, Steinheim, Germany) [14].

2.6. Sensory Evaluation. A sensory panel consisting of 25 trained men and women (10 men and 15 women) in the age range between 20 and 45 years from students and employees of the Islamic Azad University of Varamin-Pishva branch (Varamin, Iran) participated in this test. A 5-point hedonic scale, where "5" represents "most like" and "1" "most dislike," was used to assess sensory parameters, including flavor and odor.

2.7. Statistical Analysis. Each UF feta cheese treatment was produced in triplicate, and measurements were performed in triplicate. SPSS (V26) was used to analyze the collected data with a confidence level of 95% in order to identify significant differences ($p < 0.05$) among cheese treatments. Microbial analysis was performed using repeated-measures ANOVA. Aroma analysis was evaluated using one-way ANOVA. In both cases, Bonferroni's post hoc test was used to compare means. Nonparametric tests were used to analyze sensory characteristics. Four test samples were selected, and statistical analyses were carried out on the averages of the triplicate results.

3. Results and Discussion

The survival rate of probiotic bacteria in UF feta-type cheese during 60 days of cold storage period is shown in Figures 1–3. The survival rate of probiotic bacteria showed a decreasing trend. The results of statistical analysis showed that the effects of treatment and storage time on the survival of both bacteria were significant ($p < 0.05$). The greatest microbial loss occurs from the 1st day to the 10th day, due to the shock to the bacterial cell to adapt to the environment. After the relative adaptation, the survival of the bacteria is much higher until the end of the storage period. This finding agreed with the results obtained by Dinakar and Mistry [15]. These researchers found that *Bifidobacterium bifidum* maintains its viability until the end of the storage period to an acceptable population after being relatively adapted to the environmental conditions and the initial drop of the bacterial population [15]. It has been assumed that the lag phase allows the adaptation required for bacterial cells to begin to exploit new environmental conditions. This process could include the repair of macromolecular damage that accumulated during the stationary phase and the synthesis of cellular components necessary for growth [16]. In the case of *Enterococcus faecium*, unlike *Bifidobacterium bifidum*, a sudden drop does not occur from day 1 to day 10, and the decrease in viability continues nonstop and nonstepwise. However, its final drop on the 60th day is significantly more than *Bifidobacterium bifidum*, so on this day, the *Bifidobacterium bifidum* population decline is 93.6% and the percentage of *Enterococcus faecium* population decline is 95.1. Therefore, it seems that *Bifidobacterium bifidum* after the initial sudden drop from day 1 to day 10 has adapted better and shows more resistance for survival, and if the base of probiotic cheese is at least 10^7 CFU/g of live cells, this condition regarding *Bifidobacterium bifidum* bacteria ends before the 10th day of storage. However, in the case of *Enterococcus faecium* bacteria, it ends before the 30th day of storage. On the other hand, pH, microstructure, acidity level, and suitable Eh are all considered factors for maintaining the viability of probiotic bacteria in UF cheese. Compared to *Bifidobacterium bifidum*, *Enterococcus faecium* has higher resistance and adaptability to the stresses in the environment. These microorganisms guarantee the production of a high quantity of lactic acid, which contributes to rapid milk acidification and the production of several flavor and texture compounds [2]. Gobbetti et al. reported that the survival of probiotics in samples with a concentration of more than 4% salt was disturbed, and the population of probiotic bacteria decreased [17]. Dinakar and Mistry showed that cheddar cheese was able to maintain an acceptable population of *Bifidobacterium bifidum* species until the end of the storage period, which agreed with the results of the present study [15]. Shahab Lavasani reported that bifidobacteria had a satisfactory viability in the feta cheese during 60 days of refrigerated storage [8].

3.1. Aroma Characteristic. Flavor is one of the important sensations that is created by food in the mouth and is understood with the help of the sense of taste, smell, touch, and the general receptors of pain, touch, and temperature in the mouth.

3.2. Changes in Acetaldehyde (μg/g). The evaluation of acetaldehyde changes in UF white cheese showed that the effects of treatment, time, and the interaction of treatment × time on the amount of acetaldehyde are significant ($p < 0.01$), and according to Figure 4, the amount of acetaldehyde in all treatments increased with time and the intensity of the increase in treatment D containing *Bifidobacterium bifidum* and *Enterococcus faecium* were more severe. The reason for the increase in acetaldehyde production in treatment D has more metabolic activity compared to other treatments. Acetaldehyde is responsible for the piquant fruit flavor in UF cheese and is produced by lactose metabolism or ethanol oxidation [18]. Threonine decomposition also produces acetaldehyde and aldehydes with side branches such as 2-methylbutanol and 3-methylbutanal are the result of leucine and isoleucine metabolism. With regard to aromatic aldehydes, these compounds are mainly formed starting from α-keto acids derived from the benzaldehyde released by the spontaneous oxidation of tryptophan and phenylalanine [7]. In general, the low amount of aldehyde compounds is attributed to the presence of reductases from cheese microbiota. Rasouli Pirouzian et al. reported that in the cheese produced with *E. faecalis* and *E. faecium strains*, lipolysis rate was higher and flavor properties were improved [5].

3.3. Changes in Deacetyl (μg/g). The effects of treatment, time, and the interaction of treatment × time on the amount of diacetyl (μg/g) are significant ($p < 0.05$). The changes in diacetyl (μg/g) (during the 60-day storage period) are shown in Figure 5. The amount of diacetyl in treatment A increased

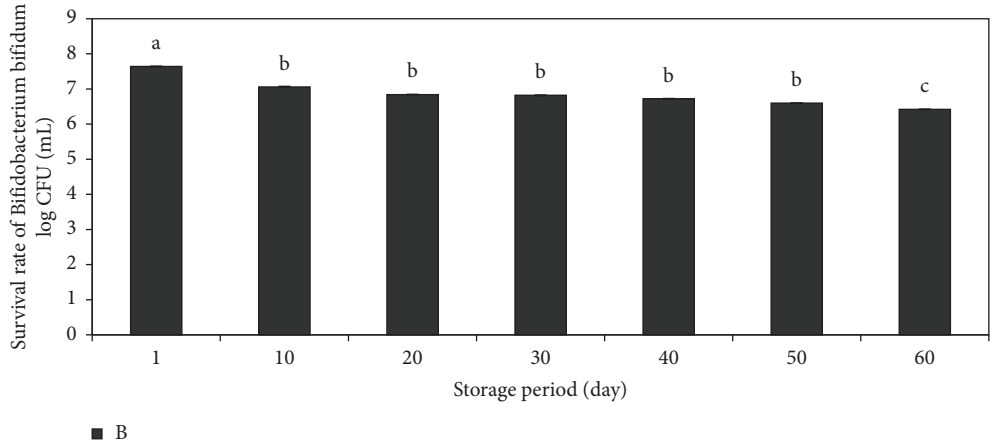

Figure 1: Changes in the survival rate of *Bifidobacterium bifidum* log CFU/g of UF cheese* during the 60-day storage period. *Treatment B: UF cheese containing *Bifidobacterium bifidum*. [a–c]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

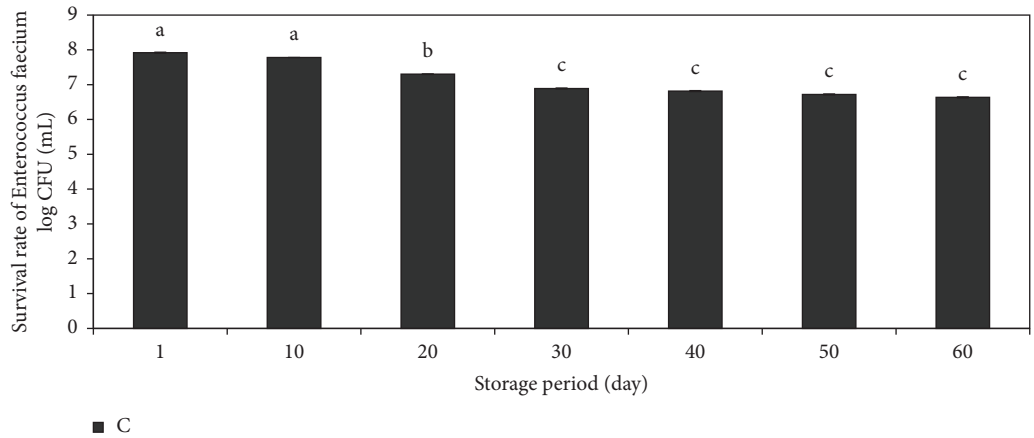

Figure 2: Changes in the survival rate of *Enterococcus faecium* log CFU/g of UF cheese* during the 60-day storage period. *Treatment C: UF cheese containing *Enterococcus faecium*. [a–c]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

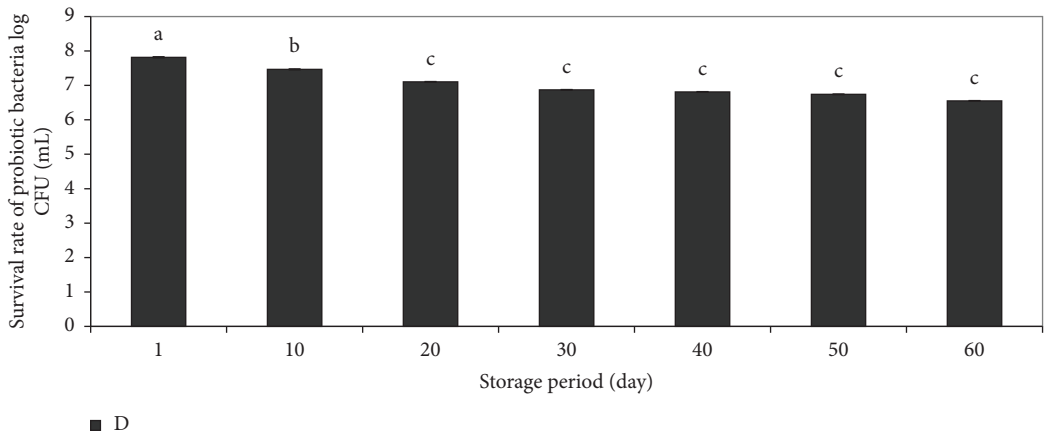

Figure 3: Changes in the survival rate of probiotic bacteria log CFU/g of UF cheese* during the 60-day storage period. *Treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a–c]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

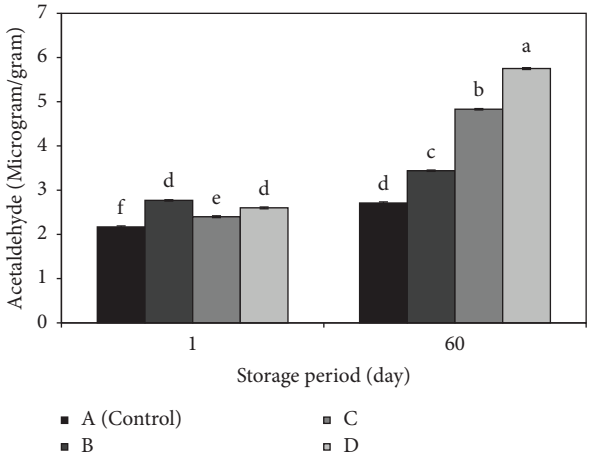

Figure 4: Changes in acetaldehyde (μg/g) content of UF cheese* during the 60 days of storage. *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*, and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a–f]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

during the 60-day storage period, while the other treatments showed a decreasing trend. The reduction of diacetyl to acetylene caused a decrease in the amount of diacetyl in the treatments except for the control treatment, and in the control treatment that did not have a probiotic strain, the process of reducing diacetyl to acetylene did not take place during the storage period; as a result, the concentration of diacetyl increased during the storage period.

3.4. Changes in Acetoin (μg/g). The effects of treatment, time, and the interaction of treatment × time on the amount of acetoin (μg/g) are significant ($p < 0.05$). The changes in acetoin (μg/g) (during the 60-day storage period) are shown in Figure 6. As can be seen in Figure 4, with the exception of the control sample, there is a decrease in the amount of acetoin in other treatments during the 60-day shelf life. The reduction of diacetyl to acetoin increased the amount of acetoin, and further reduction of acetoin caused the formation of butane-2,3-diol and subsequently butanone and butanol [13]. In other cheese variants, except for the control sample, the reduction of diacetyl to acetoin took place more intensively.

3.5. Changes in Acetic Acid (μg/g). The effects of treatment, time, and the interaction of treatment × time on the amount of acetic acid (μg/g) are significant ($p < 0.05$). The changes in acetic acid (μg/g) (during the 60-day storage period) are shown in Figure 7. With the exception of the control treatment, the amount of acetic acid has decreased in other treatments during the 60-day storage period. The reason for the increasing content of acetic acid in control cheese was attributed to lactate fermentation since lactose can be present even in mature cheese [8]. Acetic acid is one

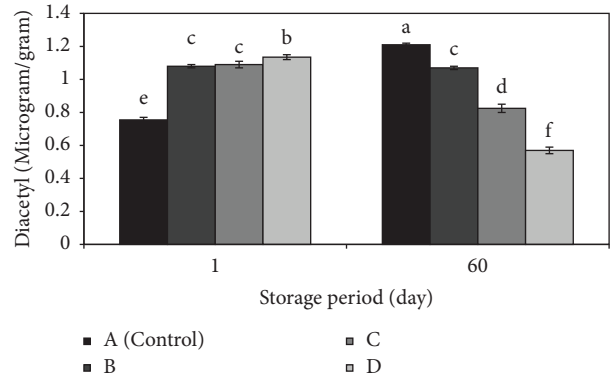

Figure 5: Changes in diacetyl (μg/g) content of UF cheese* during the 60-day storage period. *Treatment A: (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*; and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a–f]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

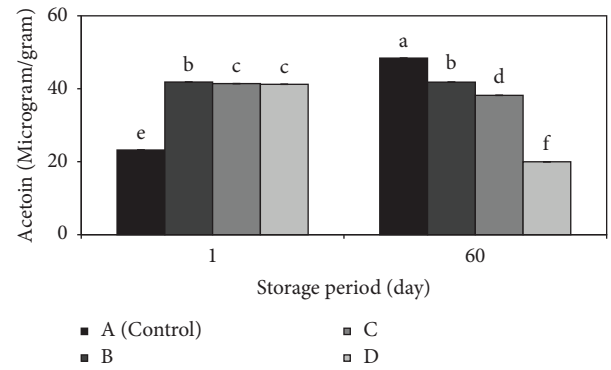

Figure 6: Changes in acetoin (μg/g) content of UF cheese* during the 60-day storage period. *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*, and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a–f]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

of the most important compounds in the flavor of UF cheese. Acetic acid is the result of the decomposition of lactose and amino acids [19], it has a very low flavor threshold, and its characteristic taste is vinegary and sour and is the precursor of lactones, alcohols, lactones, and esters [9]. So, its reduction in experimental cheeses B, C, and D containing probiotic strains is due to its participation as a precursor in the production of methyl ketones, alcohols, lactones, and esters. Acetic acid in high concentration in blue cheese has been identified by other researchers [20].

Acetic acid forms during the early stages of ripening and is probably a product of citrate or lactate fermentation or of amino acid catabolism by bacteria [8]. Shahab Lavasani reported that the kind and quality of milk and the

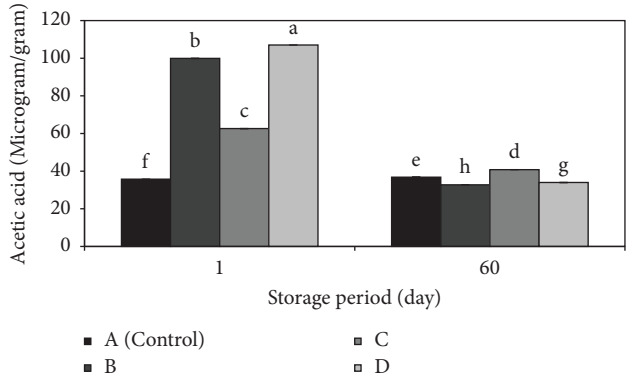

Figure 7: Changes in acetic acid (μg/g) content of UF cheese* during the 60-day storage period. *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*, and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a–f]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

conditions of manufacturing and ripening are the most important factors affecting the concentration of acetic acid in Lighvan cheese [7].

3.6. Changes in FFA Composition. Milk fat hydrolysis during cheese manufacture and ripening is due to the endogenous milk lipase, the lipolytic enzymes of starter and nonstarter bacteria, lipases from psychrotrophic bacteria, and exogenous enzyme preparations. Fatty acids can be further converted to methyl ketones and thioesters, which have been implicated as cheese flavor compounds. The cheeses with adjunct starters, in general, exhibited significantly ($p < 0.01$) higher levels of lipolysis index by the progress of ripening compared to the control cheese. Cheese made with *E. faecalis* and *E. faecium* strains involved the highest level of FFA. These results indicated that adjunct enterococci contributed to lipolysis in cheese [5]. During ripening, the lipolysis results in increased free fatty acid (FFA) concentrations, which in turn affects the textural and sensory characteristics of cheese samples. Hydrolysis of fat is especially important in soft cheeses [21].

3.7. Short-Chain Free Fatty Acids (SCFFAs)

3.7.1. Butyric Acid ($C_{4:0}$). Among the short-, medium-, and long-chain free fatty acids, the effect of treatment, except for arachidic acid $C_{20:0}$, the effect of time, and the interaction effect of treatment × time were observed ($p < 0.01$). The amount of butyric acid $C_{4:0}$ in all experimental cheeses, except treatment B containing the probiotic *Bifidobacterium bifidum* strain, increased to some extent during the storage period (Table 1). In the experimental cheeses containing *Enterococcus faecium* and the combination of *Enterococcus faecium* and *Bifidobacterium bifidum*, the amount of butyric acid increased during the storage period due to the higher lipolytic activity, which can be attributed to the effect of lipase enzyme on the hydrolysis of triglycerides. The formation of short-chain free fatty acids is mostly attributed to the specific action of natural lipoprotein lipases and natural microflora lipases of raw milk on free fatty acids located in the Sn-1 and Sn-3 positions of the triglyceride chain. The high percentage of butyric acid in white brine cheese shows that fat hydrolyzing enzymes have acted selectively [22]. The results of this research were consistent with the results of Jaeggi et al., who stated that the amount of butyric acid was significantly higher in all cheeses made from milk-containing somatic cells, more than in cheeses made from milk-containing medium and low somatic cells [23]. The high level of free C4:0 in raw cow's milk originates from β-hydroxybutyric acid produced by the fermentation of carbohydrates by rumen microorganisms, which is transported through the bloodstream to the mammary gland to be reduced to free C4:0 [24]. Santillo et al. stated that rennet paste containing probiotics showed a lipase activity 2-fold greater than that displayed by traditional rennet [25]. Bifidobacteria can produce mainly acetate and formate by using the fermentation pathway. Because of the beneficial health effects of probiotics, prebiotics, and their metabolites, they are used as food supplements or the SCFFA producers, and bifidobacteria/lactobacilli are added to dairy products, such as in cheese and yogurt [26]. Therefore, the amount of butyric acid increased according to the enzymatic activities and fermentation pathway of *Bifidobacterium bifidum*.

3.7.2. Caproic Acid ($C_{6:0}$). Caproic acid ($C_{6:0}$) in experimental cheeses containing *Bifidobacterium bifidum* increased until the end of day 30 of the storage period and then decreased until the end of the storage period (Table 1). The reason for the reduction of caproic acid is the conversion of this short-chain fatty acid into compounds effective in flavor, and the reason for its increase is the selective effect of natural milk lipases. The results of the present research were consistent with the results obtained by Yazdanpanah et al. who stated that the decrease in the amount of fat in the samples is due to the conversion into free fatty acids, and by protein hydrolysis, new links are created by protein networks [27]. Kandarakis et al. showed that the ripening period was the most important factor in the increase of fatty acids in white brine cheese [28].

3.7.3. Caprylic Acid ($C_{8:0}$). The amount of caprylic acid ($C_{8:0}$) in the control cheese increased until the 30th day of the storage period and then decreased until the end of the storage period. The amount of caprylic acid in treatment B containing *Bifidobacterium bifidum* decreased until day 30 of the storage period and then increased until day 60 of the storage period (Table 1). With the addition of the lipase enzyme and over time storage period, the amount of volatile fatty acids increases significantly compared to medium-chain and long-chain fatty acids, and the reason for this is the specificity of this enzyme for the Sn-3 position of fatty triglycerides [29]. The reason for the reduction of caprylic acid is the conversion of this short-chain fatty acid to

Table 1: Changes in short-chain free fatty acids (SCFFAs) of probiotic Iranian UF cheese during the 60-day storage period.

SCFFAs g/100 g	Treatments*	Storage period (days)		
		1	30	60
$C_{4:0}$	A	3.89 ± 0.03^{Cb}	6.25 ± 0.17^{Bd}	16.64 ± 0.07^{Aa}
	B	14.74 ± 0.03^{Ca}	13.76 ± 0.06^{Ba}	11.445 ± 0.075^{Ac}
	C	4.265 ± 0.025^{Cc}	7.19 ± 0.06^{Bb}	7.88 ± 0.06^{Ad}
	D	4.02 ± 0.075^{Cd}	6.50 ± 0.05^{Bc}	12.03 ± 0.07^{Ab}
$C_{6:0}$	A	2.83 ± 0.02^{Cb}	6.45 ± 0.025^{Bc}	17.47 ± 0.045^{Aa}
	B	5.19 ± 0.02^{Ca}	14.93 ± 0.03^{Ba}	5.24 ± 0.065^{Ac}
	C	2.66 ± 0.02^{Cc}	13.465 ± 2.48^{Ba}	13.57 ± 0.035^{Ab}
	D	2.39 ± 0.03^{Cd}	7.47 ± 0.025^{Bb}	3.63 ± 0.025^{Ad}
$C_{8:0}$	A	1.59 ± 0.025^{Cb}	3.53 ± 0.02^{Aa}	1.97 ± 0.02^{Bd}
	B	1.455 ± 0.025^{Bd}	0.854 ± 0.025^{Cd}	3.495 ± 0.025^{Ab}
	C	1.77 ± 0.02^{Ca}	2.09 ± 0.02^{Bc}	7.07 ± 0.03^{Aa}
	D	1.52 ± 0.03^{Cc}	2.15 ± 0.03^{Bb}	2.48 ± 0.03^{Ac}

$^{a-d}$Means in the same column shown with different letters are significantly different ($p < 0.05$). $^{A-C}$Means in the same row shown with different letters are significantly different ($p < 0.05$). *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*; and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*.

compounds effective in flavor; besides, its increase is due to the selective effect of natural milk lipases. These FAs are responsible for the specific aroma of milk and its derivatives "Goaty" flavor mainly derive from the presence of SCFAs, such as C6:0, C8:0, and C10:0 acid [30]. SCSFA (short-chain saturated fatty acid) plays a vital role in the flavor composition of different products, contributing to their uniqueness. Besides, the consumption of SCSFA is positively associated with health benefits, such as reducing the risk of developing cancer, cardiovascular disease, and gastrointestinal disorders [31].

3.8. Medium-Chain Free Fatty Acids (MCFFAs). Most of the FAs were under the influence of the period and interactions between diet and period. Cheeses were made during different lactation stages, influencing different chemical compositions and FAs. These fluctuations during lactation affected the decreased proportions of C8:0 and C10:0 during lactation. These fluctuations could reflect an increased uptake of dietary FAs by adipose tissue after the lactation peak of cows [30].

3.8.1. Capric Acid ($C_{10:0}$). The amount of capric acid ($C_{10:0}$) in all experimental cheeses, except treatment B containing *Bifidobacterium bifidum*, decreased until the end of the storage period, and in treatment B, it decreased until the 30th day of the storage period and then increased until the end of the storage period (Table 2). In a similar study, it was shown that the amount of volatile free fatty acids and the total amount of free fatty acids increased significantly with the addition of pregastric lipase enzyme to white brine cheese [32]. Capric acid decreased in all treatments due to the conversion of those effective compounds in aroma and flavor; however, in treatment B, capric acid decreased, and its reason is converting capric acid to aroma and flavor compounds. Then, because of enzyme activities and suitable conditions for the activity of *Bifidobacterium bifidum* and starter culture and hydrolysis of triglycerides, the amount of capric acid increased. Aminifar and Emam-Djomeh reported that capric acids were the main free fatty acids in Orgu cheese [33].

3.8.2. Lauric Acid ($C_{12:0}$). The amount of lauric acid ($C_{12:0}$) in the control cheese decreased until the end of the storage period; however, in all experimental cheeses, the amount of lauric acid decreased until the 30th day of the storage period and then increased until the 60th day of the storage period (Table 2). An increase in lauric acid was attributed to a decrease in the moisture of the curd and an increase in the fat content of the curd. The reason for the decrease in lauric acid, especially at the end of the first month of the storage period, was its decomposition and destruction, especially in treatments containing probiotic strains. The results of this research did not agree with the results obtained by other researchers [1]. They found that lauric acid increased during the storage period of white brine cheese. Efthymiou and Mattick reported that the high amount of lauric acid and free fatty acids are responsible for the rancid flavor in feta cheese [34].

3.8.3. Myristic Acid ($C_{14:0}$). The amount of myristic acid ($C_{14:0}$), with the exception of treatment B containing *Bifidobacterium bifidum*, decreased in all experimental cheeses until the end of the storage period. In treatment B, the amount of myristic acid decreased until the 30th day of storage and then increased until the end of the storage period (Table 2). The main reason for the reduction of myristic acid is its decomposition and transformation into other products effective in flavor and aroma. Also, the loss of moisture in treatment B and the increase in fat concentration per mass unit caused the increase of myristic acid at the end of the second month of the storage period. Some researchers reported the increase of myristic acid until the end of the 45th day of the ripening period of white brine cheese and then its decrease until the end of the 90-day ripening period, which did not agree with the results of this research [1]. Among

Table 2: Changes in medium-chain free fatty acids (MCFFAs) of probiotic Iranian UF cheese during the 60-day storage period.

MCFFAs g/100 g	Treatments*	Storage period (days)		
		1	30	60
$C_{10:0}$	A	3.70 ± 0.02^{Ab}	3.15 ± 0.04^{Ba}	1.785 ± 0.025^{Cd}
	B	2.825 ± 0.025^{Ad}	2.295 ± 0.025^{Bd}	2.825 ± 0.025^{Ca}
	C	3.82 ± 0.06^{Aa}	2.825 ± 0.025^{Bc}	1.855 ± 0.025^{Cc}
	D	3.54 ± 0.25^{Ac}	2.915 ± 0.025^{Bb}	2.780 ± 0.01^{Cb}
$C_{12:0}$	A	3.97 ± 0.02^{Aa}	3.24 ± 0.03^{Ca}	2.625 ± 0.025^{Bd}
	B	3.26 ± 0.02^{Ab}	2.935 ± 0.035^{Cb}	3.10 ± 0.02^{Ba}
	C	4.11 ± 0.03^{Aa}	2.20 ± 0.04^{Cd}	2.865 ± 0.025^{Bc}
	D	3.955 ± 0.025^{Aa}	2.34 ± 0.03^{Cc}	3.02 ± 0.03^{Bb}
$C_{14:0}$	A	11.55 ± 0.02^{Ad}	9.735 ± 0.035^{Bb}	7.615 ± 0.035^{Cd}
	B	9.74 ± 0.03^{Ab}	7.545 ± 0.045^{Bd}	9.50 ± 0.040^{Ca}
	C	11.745 ± 0.035^{Aa}	8.8658 ± 0.035^{Bc}	8.08 ± 0.04^{Cc}
	D	11.585 ± 0.035^{Ac}	9.81 ± 0.03^{Ba}	8.71 ± 0.04^{Cb}

$^{a-d}$Means in the same column shown with different letters are significantly different ($p < 0.05$). $^{A-C}$Means in the same row shown with different letters are significantly different ($p < 0.05$). *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing Enterococcus faecium; and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*.

medium-chain FFA, lauric acid had the lowest concentration in all cheese samples. However, myristic acid was the predominant medium-chain FFA at the beginning of the ripening period. After 120 days of ripening, lauric and myristic acid were dominant among medium-chain FFA. Despite the quantitative importance of medium- and long-chain FFA, they are not the main factor for the cheese flavor [35].

3.9. Long-Chain Free Fatty Acids (LCFFAs). Generally, the total FFA contents found in the cheeses were significantly higher in cheeses C and D. This may be related to the higher lipolytic activity of enterococcal strains in these cheeses. Compared to many cheese varieties, the total FFA content of UF cheese was markedly lower [35].

3.9.1. Palmitic Acid ($C_{16:0}$). The amount of palmitic acid in all experimental cheeses, except treatment B containing *Bifidobacterium bifidum*, decreased until the end of the storage period; however, the amount of palmitic acid in treatment B decreased until the end of the first month and then increased until the end of the storage period (Table 3). Palmitic acid is the most saturated fatty acid among free fatty acids. The selective effect of lipase enzyme, especially on long-chain fatty acids in the Sn-3 position, is the reason for the increase of palmitic acid and the destruction and decomposition of palmitic acid produced as a result of enzyme activity, the reason for its decrease.

3.9.2. Stearic Acid ($C_{18:0}$). Stearic acid concentration of all experimental cheeses, except treatment B containing *Bifidobacterium bifidum*, decreased until the end of the storage period; however, the amount of stearic acid of treatment B decreased slightly until the end of the first month and then increased until the end of the storage period (Table 3). At the beginning of the storage period, the lipolytic activity is intense, especially at the beginning of the ripening period, and the low concentration of salt in the curd leads to more lipase enzyme activity, which causes an increase in the concentration of stearic acid, and consequently, the released fatty acids are broken down into other compounds which reduced the amount of stearic acid until the end of the first month of the storage period. Katsiari et al. reported that stearic acid was hydrolyzed to the same extent in all treatments during the storage of UF cheese [36]. El-Metwally et al. reported that the most abundant saturated fatty acids were palmitic acid (C16:0) followed by stearic (C18:0) acid and myristic acid (C14:0) [37].

3.9.3. Oleic Acid ($C_{18:1}$). The amount of oleic acid in all experimental cheeses, except treatment B containing *Bifidobacterium bifidum*, increased during the storage period; however, the trend of changes in the amount of oleic acid in treatment B during the storage period showed a decrease (Table 3). The reason for the increase in oleic acid is the increase in storage time and the more intense lipolytic activity in the samples that contain *Enterococcus faecium* strain, which is the reason for its degradation and breakdown into other compounds effective in flavor. Among the unsaturated fatty acids, oleic acid had the highest concentration in terms of quantity. The results obtained from this research are in agreement with the results obtained by Katsiari et al. [36]. These researchers reported that the predominant unsaturated fatty acid in feta cheese is oleic acid. Among USFAs (unsaturated fatty acids), oleic acid (C18:1) had the highest concentration in all cheese treatments. The role of USFA in the lowering of harmful LDL cholesterol is scientifically established. Nutritionists suggest taking half of oil-derived calories from oleic acid (omega-9) to minimize the dangers of cardiovascular diseases [37].

3.9.4. Linoleic Acid ($C_{18:2}$). The trend of linoleic acid changes is such that in treatment B (UF cheese containing *Bifidobacterium bifidum*), the amount of linoleic acid increased until the end of the 60-day storage period, and in treatment A (control), the amount of linoleic acid increased until the 30th day of the storage period and then decreased

Table 3: Changes in long-chain free fatty acids (LCFFAs) of probiotic Iranian UF cheese during the 60-day storage period.

LCFFAs(g/100 g)	Treatments*	Days of storage period		
		1	30	60
$C_{16:0}$	A	32.17 ± 0.02^{Ac}	28.175 ± 0.025^{Bb}	20.415 ± 0.025^{Cd}
	B	26.865 ± 0.025^{Ad}	22.835 ± 0.025^{Bd}	27.85 ± 0.02^{Ca}
	C	32.275 ± 0.015^{Ab}	25.585 ± 0.015^{Bc}	23.560 ± 0.02^{Cc}
	D	33.055 ± 0.025^{Aa}	28.980 ± 0.01^{Ba}	26.854 ± 0.025^{Cb}
$C_{18:0}$	A	8.475 ± 0.015^{Ab}	6.25 ± 0.02^{Bb}	4.675 ± 0.015^{Cd}
	B	6.265 ± 0.025^{Ad}	5.12 ± 0.02^{Bd}	5.915 ± 0.025^{Cb}
	C	8.605 ± 0.025^{Ac}	5.895 ± 0.025^{Bc}	4.76 ± 0.03^{Cc}
	D	8.97 ± 0.02^{Aa}	6.73 ± 0.02^{Ba}	5.965 ± 0.015^{Ca}
$C_{18:1}$	A	19.80 ± 0.02^{Ab}	19.06 ± 0.04^{Ba}	13.65 ± 0.02^{Cd}
	B	16.57 ± 0.02^{Ad}	16.32 ± 0.03^{Bc}	16.555 ± 0.025^{Cb}
	C	19.50 ± 0.03^{Ac}	15.45 ± 0.02^{Bd}	14.09 ± 0.03^{Cc}
	D	20.05 ± 0.03^{Aa}	18.775 ± 0.015^{Bb}	19.305 ± 0.035^{Ca}
$C_{18:2}$	A	2.825 ± 0.015^{Ba}	3.145 ± 0.015^{Ba}	2.455 ± 0.025^{Ad}
	B	2.425 ± 0.025^{Bc}	2.725 ± 0.025^{Bb}	2.87 ± 0.02^{Ab}
	C	2.665 ± 0.025^{Bd}	2.275 ± 0.025^{Bd}	2.62 ± 0.04^{Ac}
	D	2.80 ± 0.02^{Bb}	2.615 ± 0.025^{Bc}	4.09 ± 0.03^{Aa}
$C_{18:3}$	A	0.055 ± 0.005^{Cc}	0.24 ± 0.01^{Ac}	0.045 ± 0.005^{Bc}
	B	0.085 ± 0.005^{Ca}	0.25 ± 0.01^{Ac}	0.355 ± 0.015^{Ba}
	C	0.05 ± 0.000^{Cc}	0.265 ± 0.015^{Ab}	0.075 ± 0.005^{Bc}
	D	0.065 ± 0.005^{Cb}	0.290 ± 0.01^{Aa}	0.215 ± 0.025^{Bb}
$C_{20:0}$	A	0.365 ± 0.015^{Aa}	0.19 ± 0.01^{Ba}	0.001 ± 0.000^{Ca}
	B	0.255 ± 0.015^{Aa}	0.1140 ± 0.096^{Ba}	0.0450 ± 0.005^{Ca}
	C	0.235 ± 0.015^{Aa}	0.1350 ± 0.005^{Ba}	0.22 ± 0.02^{Ca}
	D	0.255 ± 0.015^{Aa}	0.1750 ± 0.005^{Ba}	0.1150 ± 0.025^{Ca}

[a-d]Means in the same column shown with different letters are significantly different ($p < 0.05$). [A–C]Means in the same row shown with different letters are significantly different ($p < 0.05$). *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*; and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium.*

until the end of the period. Treatment C (UF cheese containing *Enterococcus faecium*) and treatment D (UF cheese containing *Enterococcus faecium* and *Bifidobacterium bifidum*) showed a decreasing trend in terms of the amount of linoleic acid up to the thirtieth day of the storage period and then an increasing trend until the end of the storage period (Table 3). The increase in the amount of linoleic acid is due to the selective effect of the lipase enzyme on long-chain fatty acids in the Sn-3 position, and the main reason for the decrease in linoleic acid is its degradation and decomposition into compounds effective in aroma and taste. The results of this study agreed with the results obtained by other researchers who investigated the effect of the lipolysis process on changing the composition of free fatty acids [1]. It is also well known that linoleic acid (C18:2—omega-6) and α-linolenic acid (C18:3—omega-3) have special importance in healthy nutrition [37].

3.9.5. Linolenic Acid ($C_{18:3}$). The amount of linolenic acid in treatments A (control), D (UF cheese containing *Enterococcus faecium* and *Bifidobacterium bifidum*), and C (UF cheese containing *Enterococcus faecium*) increased during the 60-day storage period from the beginning to the 30th day of the storage period. Then, a decreasing trend was observed until the end of the 60-day storage period, and in the case of treatment B (UF cheese containing *Bifidobacterium bifidum*), the amount of linolenic acid increased until the end of the storage period (Table 3). The increase in the amount of linolenic acid is attributed to the occurrence of lipolysis and the release of free fatty acids from the structure of triglycerides by enzymes of natural milk origin, and the decrease in the amount of linolenic acid can be related to the decrease in the amount of protein as a result of proteolysis of proteins and lipolysis of fat. Lin et al. reported that there is a direct relationship between the amount of protein and the production of conjugated linoleic acid (CLA) [38]. Their research showed the increasing effect of proteins (during the protonation process) in increasing the formation of CLA. On the other hand, the decrease in the amount of linolenic acid is attributed to its decomposition and destruction during the storage period, and its transformation into compounds is effective in creating aroma and flavor.

3.9.6. Arachidic Acid ($C_{20:0}$). The amount of arachidic acid in experimental cheeses A (control), B (UF cheese containing *Bifidobacterium bifidum*), and D (UF cheese containing *Enterococcus faecium* and *Bifidobacterium bifidum*) decreased during the 60-day storage period; however, the amount of arachidic acid in treatment C (UF cheese containing *Enterococcus faecium*) decreased until the 30th day of the storage period and then increased until the end of the 60-day storage period (Table 3). Arachidonic acid can be obtained from the hydrogenation of unsaturated fatty acid. The reason for the reduction of arachidonic acid is the

change and transformation into effective compounds in the aroma and flavor of cheese, and also, the reduction of the amount of arachidonic acid can be related to the reduction of the amount of protein, as a result of the proteolysis of proteins and lipolysis of fats by bacterial protease and lipase enzymes [38]. The reason for its increase is the removal of moisture from the curd and the increase in concentration per volume unit and the occurrence of lipolysis.

It is widely accepted that fatty acids directly participate in the final flavor characteristics of cheese types or indirectly act as precursors of some aroma components. C_2 to C_{10} alkanoic acids have a major effect on the flavor of cheeses. Vitova et al. reported that free fatty acids are related to the flavor characteristics of ewe's milk cheese and that volatile fatty acids are a major contributor to the flavor of Swiss cheese [9]. However, the relationship between the composition of total free fatty acids and the final flavor of cheese has not been fully proven yet. Although the appearance of free fatty acids in a complex system such as cheese should generally be considered the net result of several possible processes (such as enzymatic hydrolysis of existing glycerides, biosynthesis carried out by microflora, and nonenzymatic oxidation of long-chain unsaturated fatty acids), hydrolytic action of lipase is probably the main way of formation of free fatty acids due to the optimal pH range of cheeses. The rate of lipolysis may also be affected by lipid structure via increasing the interface for lipase activity [14]. The origin of lipase in cheese is from milk, nonstarter microorganisms, starter microorganisms, or possibly from rennet. The changes in the amount of short-, medium- and long-chain fatty acids are presented in Tables 1–3. As can be seen from these tables, the concentration of some fatty acids changed slightly during the storage period, and among the saturated fatty acids, palmitic acid had the highest concentration, and among unsaturated fatty acids, oleic acid had the highest concentration. The change in the amount of fatty acids can be due to the balance between the creation of fatty acids from milk fat and its breakdown into other aromatic compounds.

3.10. Sensory Evaluation

3.10.1. Flavor Characteristics. The sensory score of flavor increased during the 60-day storage period. The lowest flavor sensory score was related to treatment B (UF cheese containing *Bifidobacterium bifidum*) on the first day of storage, and the highest flavor sensory score was related to treatment D (UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*) on day 60 of the storage period (Figure 8). Proteolytic probiotic strains such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* can be effective in creating flavor during cheese ripening through carbohydrate metabolism, proteolysis, and a small amount of lipolysis. These enzymes hydrolyze casein and produce large and medium peptides. These peptides may later be broken down into small peptides and free amino acids by the proteolytic enzymes obtained from the microflora of starter bacteria, nonlactic acid bacteria, and probiotics, which are the most important factors in creating the flavor of cheese [39].

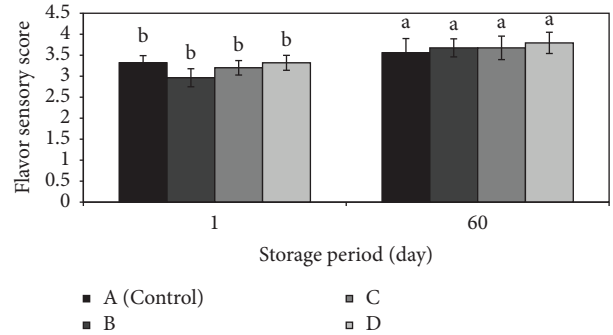

Figure 8: Changes in flavor sensory score of UF cheese* during the 60-day storage period. *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*; and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a-b]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

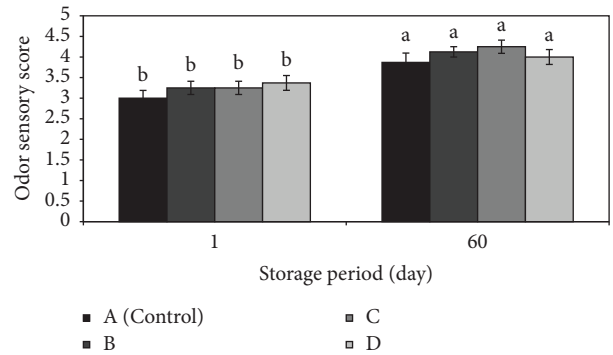

Figure 9: Changes in odor sensory score of UF cheese* during the 60-day storage period. *Treatment A (control): UF cheese without probiotic bacteria; treatment B: UF cheese containing *Bifidobacterium bifidum*; treatment C: UF cheese containing *Enterococcus faecium*; and treatment D: UF cheese containing *Bifidobacterium bifidum* and *Enterococcus faecium*. [a-b]Means with different small letter superscripts indicate significant difference ($p < 0.05$).

Lipolysis of fat and increasing the amount of free fatty acids and subsequently their biochemical reactions lead to strengthening the aroma and flavor of cheese [40]. The results of the present research were in agreement with the results obtained by Mahdavipour et al. [41]. These researchers found that probiotic strains have a positive effect on the flavor characteristics of cheese [41].

3.10.2. Odor Characteristics. The odor sensory score of experimental cheeses increased during the 60-day storage period. The highest score was related to treatment C (UF cheese containing *Enterococcus faecium*), and the lowest score was related to treatment A (control) (Figure 9). Due to the different speeds of the lipolysis process and the production of compounds affecting the aroma, changes in the intensity of the aroma were observed with increasing storage time in different treatments. The intensity and weakness of

the production of effective compounds in aroma are different in different treatments; however, according to sensory evaluators, treatment C has the optimum concentration of effective compounds in aroma and was more acceptable. The results of this research did not agree with the results of other researchers who stated that the sensory score of Lighvan enzyme-modified cheeses decreased with increasing storage time [42].

4. Conclusion

The survival rate of probiotic strains decreased during the 60-day storage period; however, all treatments had more than 10^6 CFU/g probiotic bacteria at the end of the storage period. Important changes in the concentration of compounds affecting the aroma occurred during the storage period, and unlike other compounds affecting the aroma, the amount of acetaldehyde increased during the 60-day storage period. A total of 12 free fatty acids were identified in Iranian probiotic UF white cheese, which had the highest concentration of palmitic acid among saturated fatty acids and oleic acid among unsaturated fatty acids. In general, according to the evaluated characteristics such as viability, aroma, lipolysis, and sensory, treatment D (Iranian UF white cheese containing a combination of *Bifidobacterium bifidum* and *Enterococcus faecium* strains) had good standard indicators and was selected as the best treatment.

Data Availability

The data supporting the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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