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*Research Article*

# The Quality and Composition of Iranian Low-Salt UF-White Cheese

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In cheese, the reduction of salt is still a challenging task, as sodium chloride exerts multiple and fundamental functions. Salt favors the drainage of the residual whey; enhances the taste and the aroma profile; regulates the texture, the final pH, and the water activity; and affects the microbial growth. Hence the impact of partial replacement of NaCl by KCl on the ripening characteristics of Iranian UF (ultrafiltration) cheese during storage was monitored. To produce low-salt cheese, different mixtures of UF white cheese were treated with NaCl : KCl ratios of (a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl by dry salting. ADV (acid degree value) results showed significant differences ($P < 0.05$) in all treatments after 15, 30, 40, and 50 days of ripening. No significant differences were observed in the GC (gas chromatography) results in the samples' free fatty acid (FFA) profile except for $C_{18:0}$ in all treatments. KCl did not affect the moisture, dry matter, fat, TN (total nitrogen)/dry matter, and WSN (water-soluble nitrogen) contents of cheeses considerably. The evaluation of force to fracture showed that there were significant differences ($P < 0.05$) between treatment (d) as a control cheese and treatments (b) and (c). Sensory evaluations showed as the concentration of KCl increased, the cheese gradually became less acceptable and treatments with higher potassium chloride content were crumblier and less firm. Results of the aroma evaluation of cheese samples showed that unlike acetaldehyde, ethanol, acetoin, and diacetyl amounts decreased significantly ($P < 0.05$) during the storage period. Results also indicated that a reduction of sodium by up to 50% did not significantly affect the quality and composition of Iranian low-salt UF-white cheese except for sensory evaluation, texture analysis, and aroma characteristics.

## 1. Introduction

Sodium chloride is generally found in the food supply, as table salt is a common and essential part of the human diet. In all mammals, including humans, sodium ions are necessary to regulate blood pressure and volume. They are also essential for controlling water flow into and out of the body's cells and the volume of fluids inside and outside those cells. Moreover, nerve impulse transmission and the metabolism of carbohydrates and proteins also require sodium. Chloride ions play an important role in the regulation of acid-base balance in the blood as well as in tissue osmolarity (water passes through the cell walls to maintain proper concentrations of various chemical entities). They are essential for the activation of specific enzymes and the formation of hydrochloride acid in the stomach, which is required for the digestive process [1]. Some estimates of the minimum adult daily requirement for sodium are as low as 25–50 mg per day (about 0.06–0.12 g of salt, which is 39% sodium) under favorable conditions [2]. The most common estimate of the minimum adult daily requirement is 200 mg of sodium (0.5 g of salt). Recently, the amount of salt in the diet has been a major concern, much of which centers on questions about dietary sodium and hypertension [1]. Concerns over a possible association between dietary sodium intake and cardiovascular disease have led to an effort

to reduce salt in processed foods [3], although the long-term health effects of high salt consumption have yet to be comprehensively demonstrated [4]. Reduction of salt in cheese is challenging because salt assists manufacturers in controlling various important cheese parameters, including the amount of final moisture content, microbial activity, viability of the starter bacteria, and residual enzymatic activity [5]. The salt content in cheese also has a direct influence on flavor and texture, with reduced salt cheeses often reported to be softer, more bitter, acidic, and pasty [6]. Besides, salt also has an indirect effect on the flavor of cheese through its influence on chymosin activity; decreasing the amount of NaCl salt in cheese accelerates chymosin action on $\beta$-casein ($\beta$-CN). Also, the hydrophobic peptides produced from the C-terminal of $\beta$-CN (e.g., $\beta$-CN f193–209) are quite bitter [7]. Some studies have supported a theory that increased intake of potassium chloride can reduce the blood pressure of hypertensive patients, even in the presence of excess sodium chloride [8]. Potassium chloride has been reported to exert a protective action against sodium-induced hypertension in animals and to reduce the blood pressure of diabetic children who have been consuming excess salt in their diets [1]. Many of those who follow sodium-restricted or healthy diets restrict the consumption of ripened cheeses because of their high levels of sodium [9,10]. Therefore, the dairy industry has begun looking for methods that would reduce the NaCl content of natural and processed cheeses, as they contain more sodium than other dairy products [11]. Reducing cheese salt concentration increases proteolysis, water activity, acidity, and bitterness while decreasing firmness and saltiness [12]. Abnormal fermentation can also take place [13, 14]. These factors make reducing the amount of sodium in cheese difficult, as its quality may be adversely affected. However, substituting some of the NaCl with KCl can help alleviate some of these challenges [12]. The widespread use of KCl as a partial substitution along with NaCl in cheese has been successful [11]. A number of studies have investigated how reducing the sodium content and partial or complete replacement of sodium with other salts would affect the Cheddar cheese quality. The effect of partial replacement of NaCl with KCl up to 60% with or without the addition of flavor enhancers was studied by Grummer [15]. It was found that the sensory panelists preferred the low-Na cheeses prepared with NaCl and KCl blends which were comparable to the control cheese (that contained only NaCl) [15]. Hence, the aim of this study was to determine lipolysis in UF-white cheese prepared with mixtures of NaCl and KCl and compare them to that of the NaCl (control) cheese.

## 2. Materials and Methods

*2.1. Cheese Making.* White cheese was made using an ultrafiltration unit (Model APV, Denmark) at the Kalber Dairy Company in Iran. Cow's milk (3.2% fat and 8% SNF) was heated to reach 72°C for 40 s, cooled to 36°C, and a UF unit was used for ultrafiltering to 36% total solids. The retentate was heated to 78°C for 40 s, homogenized at 70 bar, and cooled to 36°C. Two percent starter was mixed with the obtained retentate: *Streptococcus thermophilus* and

*Lactococcus bulgaricus* subsp. *bulgaricus* in a 30 : 70 ratio with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. An amount that decreased the pH of the retentate from 6.9 to around 5.2 was added, and the rennet was added (Microbial rennet, Chr. Hansen, Denmark) immediately at a rate of 3 g per 100 kg. The mixture was combined well and put into 450 g cans and was left to complete coagulation. The same method was used to manufacture lower-salt UF white cheese, which was salted as follows:

(a) 3% NaCl (control)

(b) 1.50% NaCl+1.50% KCl

(c) 1.00% NaCl+2.00% KCl

(d) 0.75% NaCl+2.25% KCl

In each trial, after the fresh samples had been taken, the cans were hermetically sealed and then stored at $4 \pm 1$ °C for 50 days. For each treatment, two replications were performed. Cans were opened, and samples were removed and assessed at 1, 15, 30, 40, 50, and 70 days after manufacture for the evaluation of lipolysis process. The chemical characteristics of UF cheese were evaluated during the 30-day period, because this period is the best time to consume UF cheese, and the evaluation of lipolysis until the end of the storage period was carried out due to the investigation of compounds that affect the aroma.

*2.2. Lipolysis.* Lipolysis level was assessed in cheese samples that had been stored for 1, 15, 30, 40, and 50 days by measuring the acid degree value. In addition, the determination of individual FFA by gas chromatography was performed in cheeses aged for 10, 25, 40, 55, and 70 days. The FFA content of all treatments was compared during the ripening period.

*2.3. Acid Degree Value (ADV).* The ADV was verified as described by other researchers [16]. To prepare samples, 5 g of cheese were mixed with 37.5 mL of 2% sodium citrate at 50°C in a Sorvall Omni-Mixer set at 3 for 1 min and then at setting 7 for 2 min. The ADV was verified using 35 mL samples of this extract.

*2.4. Free Fatty Acids (FFAs).* The extraction of cheese lipids and isolation of the FFA were executed by GC as described by De Jong and Badings [17]. Samples were prepared as follows: anhydrous $Na_2SO_4$ (4 g) was used to grind cheese (2.5 g), thereafter 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-1 each) were added. This mixture was separated three times with 3 ml diethyl ether/heptane (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 heptane. 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 $\mu$L sample was taken from this solution for GC determination of the FFA. Two chromatographic injections were made: one from each of the cheese extracts. 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 a capillary column Bp-21 (Length 30 m, 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 ($\geq$99% GC; Sigma, Steinheim, Germany).

### 2.5. Chemical Composition.
Samples were analyzed for moisture, dry matter, fat, TN/dry matter, and total WSN according to Aly [18].

### 2.6. Textural Analysis.
Uniaxial compression testing was performed using a texture analyzer (Hounsfield HSKS, UK) equipped with a 50 kg load cell and a Yokogawa model 3021 pen recorder, after 30 days of ripening. A 45-mm-diameter plunger was attached to the moving crosshead. Cubes ($3 \times 3 \times 3$ cm) from each cheese at 4°C were placed on a small dish, covered with an air-tight, plastic-wrap adhesive membrane and allowed to equilibrate to assay temperature ($20 \pm 1$°C). The sample temperature was checked by inserting a small glass thermometer into the central region of each sample cube. The operating conditions were as follows: crosshead speed 50 mm min$^{-1}$, chart speed 60 mm min$^{-1}$, and chart recording range 0–10 kg. From each force-distance curve, obtained by compression of the sample to 70% in one bite, the following texture-profile parameter was determined as described by Nishinari [19]: the force (kg) required to fracture the cheese sample was that recorded at the fracture inflection (yield point), as a measure of fracturability; a lower numerical value indicates greater fracturability. Two replicate measurements were made for each cheese.

### 2.7. Sensory Analysis.
Samples of UF white cheese were cut into pieces about $3 \times 3 \times 3$ cm in size and placed on plates coded with three-digit random numbers. The pieces were tempered by holding at ambient temperature ($20 \pm 2$°C) and presented to the panelists in a random order for testing. Water was provided for mouth washing between samples. The cheeses were evaluated organoleptically after 3, 18, and 33 days of ripening by a 10-member trained panel familiar

TABLE 1: Acid degree value (meq KOH 100g$^{-1}$ fat) of UF white∗ cheese[a,b] made with NaCl or mixtures of NaCl and KCl during 50 days of storage period.

| Duration of ripening (days) | Treatments of UF* cheese | | | |
|---|---|---|---|---|
| | (a) | (b) | (c) | (d) |
| 1 | 1.36[a] | 1.36[a] | 1.38[a] | 1.39[a] |
| 15 | 1.39[d] | 1.42[a] | 1.44[b] | 1.47[c] |
| 30 | 1.43[d] | 1.45[a] | 1.48[b] | 1.51[c] |
| 40 | 1.47[d] | 1.51[a] | 1.61[b] | 1.66[c] |
| 50 | 1.50[d] | 1.55[a] | 1.65[b] | 1.70[c] |

[a]means in each row with a superscript differed significantly ($P < 0.05$).
[b]means of two trials. Means ± standard error (a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl.

with UF white cheese. Panel members evaluated cheese for appearance; body and texture; and flavor (odor and taste). Cheeses were evaluated for flavor (scale 0–40) and body and texture (scale 0–20). The lowest score for flavor was 0 for the worst sample and the highest score was 40 for the best sample; for body and texture properties, the lowest score was 0 for the worst sample and the highest score was 20 for the best sample. Panel members were also instructed to report any defects in appearance (e.g., dry, wet, cracks), body and texture (e.g., pasty and hard), or flavor detected (e.g., rancid, bitter, metallic, salty), in accordance with the ISO-IDF (2009) guide for the sensory evaluation of cheese [20].

### 2.8. Aroma Assessments.
First, 4 g of the samples were 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, ethanol, diacetyl, acetoin, and acetic acid). The concentrations of each compound were reported as the peak areas obtained from GC [21]. To prepare the internal standards, 250 mg of pure aroma compounds (acetaldehyde, ethanol, diacetyl, acetoin, and acetic acid) were 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 as follows: helium gas purity 99.9%, pressure 5 psi, column diameter 0.32 mm, and column thickness 2.1 um.

### 2.9. Statistical Analysis.
The data were statistically analyzed using a completely randomized design (CRD) with two replications. Data were subjected to analysis of variance using the SAS statistical software package [22]. A mean comparison was performed with the LSD test at the $P < 0.05$ level of significance. Chemical composition, aroma characteristics, and textural analysis of all treatments were
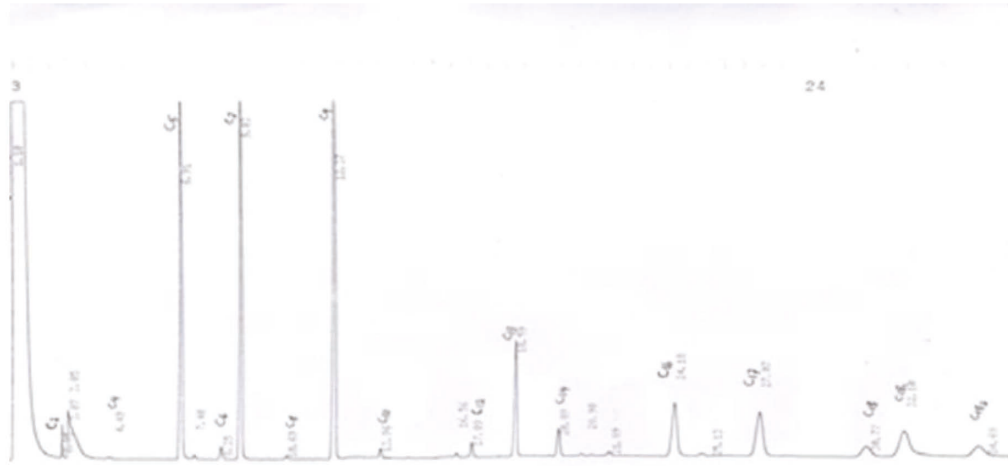
FIGURE 1: Gas chromatogram of FFA extracted from a UF white cheese (70 d old) spiked with internal FFA standards $C_{5:0}$, $C_{7:0}$, $C_{9:0}$, $C_{13:0}$, and $C_{17:0}$.

analyzed during 1, 15, and 30 days of storage period; however, ADV was analyzed during 1, 15, 30, 40, and 50 days of storage period. Free fatty acids were analyzed during 10, 25, 40, 55, and 70 days of storage period, and finally sensory analysis (flavor; body and texture) was carried out during 3, 18, and 33 days of storage period.

## 3. Results and Discussion

*3.1. Acid Degree Value.* The ADVs of UF white cheeses are presented in Table 1. ADV increase in all cheeses was observed during 50 days of storage period. This finding is consistent with the results of other investigators who found an increasing trend in ADV among all cheeses during ripening [23, 24]. On day 1 of aging, no significant differences were observed ($P < 0.05$) in ADV in any of the treatments, as none had been sufficiently affected during the short ripening period. However, as ripening continued over 15, 30, 40, and 50 days, significant differences ($P < 0.05$) in ADV emerged among all cheeses. The cheeses containing higher concentrations of KCl showed greater microbial and enzyme activities because the inhibitory effect of potassium was lower than that of sodium [24]. The highest ADV was found in treatment (c), as it had the highest potassium. The control treatment (d) had the lowest ADV among all cheeses during similar aging. The ADV for treatment (a) was the closest to the control cheese.

*3.2. Free Fatty Acids (FFAs).* Chromatographic separation of basic FFAs (Figure 1) permitted all major fatty acids to be measured in one go. Table 2 presents the mean concentrations of individual FFAs for UF white cheeses during ripening periods of 10, 25, 40, 50, and 70 days. These results demonstrated no significant differences ($P < 0.05$) in the FFA concentration among all cheeses for $C_{2:0}$, $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, and $C_{18:2}$, but the content of $C_{18:0}$ in treatment (c) differed significantly ($P < 0.05$) from treatments (a), (b), and (d) after 10, 25, and 40 days of ripening; this could be the result of more

microbial and enzyme activities. Because treatment (c) had the lowest sodium, the concentration of $C_{18:0}$ was increased. The inhibitory effect of NaCl on lipolysis was regarded as the reason for the slower rate of FFA production in the control cheese. The level of $C_{18:0}$ in treatment (b) differed significantly ($P < 0.05$) from the other treatments after 50 and 70 days, although the level of $C_{18:0}$ in treatment (a) was less than in treatments (b) and (c), and no significant difference ($P < 0.05$) was noted from the control cheese. Total FFA in control cheese was marginally lower compared to the experimental cheeses after the same aging time. These results were consistent with the results of other researchers who stated that Cheddar cheeses produced using NaCl/KCl mixtures had higher concentrations of total FFA compared to those made with similar levels of NaCl [9, 11]. Acetic acid contributes greatly to the flavor of Feta cheese [25]. The high rate of FFA release, up to the end of the ripening period, was attributed to the enzymic activity of cheese microflora by the relatively reduced sodium in cheeses. The microbial populations of traditional Feta cheese during ripening were studied by Manolopoulou [10]; it was found that microorganisms with high lipolytic activity, including yeast, nonstarter lactic acid bacteria (NSLAB), enterococci, and micrococci, attained their highest levels during the first 16 days [10]. In addition, Kandarakis et al. realized that the high temperature during the preripening period of Feta cheese favors the release of $C_{2:0}$–$C_{12:0}$ FFA [23]. The acetic acid level was between 41% and 1.13% in comparison with total FFA present in UF white cheeses after the same aging periods (Table 2). Vafopoulou et al. stated that acetic and palmitic acids were the major FFAs in Feta cheese salted only with NaCl and aged 40 and 120 days [27]. Katsiari et al.'s study on Feta cheese produced by partial substitution of NaCl with KCl showed that 40 to 44% of the total FFAs present in Feta cheeses contained acetic acid [24]. The results of this study confirm exactly the results of the above research (Table 2). This proportion is comparable to those reported by other investigators [25,28] for Feta cheese. Efthymiou (1967) reported that acetic acid comprised 28–43% of all FFAs

TABLE 2: Free fatty acids (g 100g⁻¹) of UF white* cheese[a, b] made with NaCl or mixtures of NaCl and KCl and aged for 10, 25, 40, 55, and 70 days.

| FFA | Storage periods (Day) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | | | | 25 | | | | 40 | | | | 55 | | | | 70 | | | |
| | (a) | (b) | (c) | (d) | (a) | (b) | (c) | (d) | (a) | (b) | (c) | (d) | (a) | (b) | (c) | (d) | (a) | (b) | (c) | (d) |
| C2:0 | 0.63 | 0.76 | 0.81 | 0.42 | 0.65 | 0.78 | 0.86 | 0.82 | 0.68 | 0.83 | 0.95 | 0.47 | 0.71 | 0.84 | 1.05 | 0.54 | 0.75 | 0.87 | 1.13 | 0.60 |
| C4:0 | Trace | 0.12 | Trace | Trace | Trace | 0.12 | Trace | 0.06 | Trace | 0.13 | Trace | Trace | Trace | 0.13 | Trace | Trace | Trace | 0.14 | Trace | Trace |
| C6:0 | 0.49 | 0.76 | 0.69 | 0.69 | 0.51 | 0.78 | 0.74 | 0.76 | 0.53 | 0.83 | 0.81 | 0.77 | 0.55 | 0.84 | 0.89 | 0.85 | 0.58 | 0.87 | 0.97 | 0.92 |
| C8:0 | Trace | 0.06 | Trace | Trace | Trace | 0.06 | Trace | 0.03 | Trace | 0.06 | Trace | Trace | Trace | 0.07 | Trace | Trace | Trace | 0.07 | Trace | Trace |
| C10:0 | 0.21 | 0.40 | 0.27 | 0.28 | 0.22 | 0.41 | 0.29 | 0.35 | 0.23 | 0.43 | 0.32 | 0.31 | 0.24 | 0.44 | 0.35 | 0.35 | 0.26 | 0.46 | 0.38 | 0.38 |
| TVFFA[c] | 1.33 | 2.10 | 1.76 | 1.40 | 1.38 | 2.16 | 1.89 | 2.02 | 1.44 | 2.28 | 2.08 | 1.55 | 1.50 | 2.33 | 2.29 | 1.74 | 1.59 | 2.40 | 2.48 | 1.90 |
| C12:0 | 0.49 | 0.51 | 0.57 | 4.34 | 0.51 | 0.52 | 0.61 | 0.56 | 0.53 | 0.54 | 0.67 | 4.74 | 0.55 | 0.55 | 0.74 | 5.01 | 0.58 | 0.57 | 0.81 | 5.28 |
| C14:0 | 1.04 | 1.70 | 1.37 | 5.04 | 1.08 | 1.75 | 1.47 | 1.61 | 1.12 | 1.85 | 1.62 | 5.50 | 1.17 | 1.89 | 1.79 | 5.86 | 1.23 | 1.95 | 1.94 | 6.20 |
| C16:0 | 4.89 | 6.36 | 5.27 | 8.16 | 5.06 | 6.55 | 5.65 | 6.10 | 5.25 | 6.92 | 6.21 | 9.09 | 5.47 | 7.05 | 6.85 | 10.37 | 5.75 | 7.28 | 7.48 | 11.43 |
| C18:0 | 2.03[a] | 2.10[a] | 3.47[a] | 7.21[b] | 2.10[a] | 2.16[a] | 3.70[a] | 7.76[b] | 2.17[a] | 2.28[a] | 4.11[a] | 7.96[b] | 2.26[a] | 2.33[a] | 4.49[b] | 8.81[c] | 2.37[a] | 2.40[a] | 4.84[b] | 9.54[c] |
| C18:1 | 6.37 | 6.36 | 11.91 | 13.55 | 6.57 | 6.57 | 12.68 | 9.63 | 6.80 | 6.95 | 14.11 | 15.13 | 7.07 | 7.08 | 15.40 | 17.37 | 7.42 | 7.31 | 16.53 | 19.24 |
| C18:2 | 3.85 | 3.29 | 7.84 | 10.17 | 3.97 | 3.39 | 8.35 | 5.87 | 4.10 | 3.60 | 9.28 | 11.35 | 4.26 | 3.66 | 10.14 | 13.04 | 4.47 | 3.79 | 10.90 | 14.44 |

present in mild-flavored Feta cheese samples of unknown age, and Zerfiridis and Kristoffersen (1968) found a proportion of 32% for 60-day-old Feta cheese [25, 28]. However, lower values (~20%) have also been reported [27]. The results of this study regarding the proportion of acetic acid to total FFA concentration support those of Efthymiou (1967), who indicated the importance of acetic acid as a major fatty acid constituent and as a flavor and aroma determinant in Feta cheese [25]. Starter bacteria are the crucial determinants of lipolysis that occur during the ripening of the majority of cheeses made from pasteurized milk [29]. In starter cultures, lactic acid bacteria (LAB) are the predominant microorganisms which are also observed in cheese as adventitious non-starter lactic acid bacteria (NSLAB) [30]. A considerable multiplicity of LAB possess esterolytic enzymes, but true lipolytic enzymes capable of hydrolyzing milk fat in milk and cheese are found in few species [31]. Esterase activities have been distinguished in streptococci, lactococci, and mesophilic and thermophilic lactobacilli [32]. Yet, compared to typical lipolytic bacteria, LAB, especially *Lactococcus* and *Lactobacillus* spp., have weak lipolytic activity [33]. However, because starters and NSLAB are present in cheese in high numbers over an extended ripening period, their enzymes may be responsible for the liberation of significant FFA levels in cheese [31]. Their role is important in types of cheese that do not contain secondary microorganisms with strong lipolytic activity. Among LAB, enterococci showed much more intense lipolytic activities than other species [34]. Variability in lipolysis pattern among replicate trials has also been reported (e.g., Oliszewski et al. in goats' milk cheeses) [35]. Such variations could be due to differences in the initial level of FFAs in fresh milk, which can be modulated by many factors (lactation stage; damage of milk fat globule membrane (MFGM) during milk transport allowing the accessibility of fat to the lipoprotein lipase (LPL); and during milk technological treatments, such as homogenization and heat-pressure treatment). These variations can have a great influence in internally lactic acid bacteria (LAB)-ripened cheeses. For example, from the data of the kinetics of lipolysis in Cheddar cheeses made under different conditions (starters, milk treatments, lactation stage), it can be anticipated that 72–90% of the final amount of FFAs were present at day 1 [36]. The activity of LPL also releases partial glycerides which are better substrates for most LAB enzymes and propionic acid bacteria compared to triglycerides [37]. The rate of lipolysis may also be affected by lipid structure via increasing the interface for lipase activity. In the manufacture of the Danish blue cheese Danablu, the cream is homogenized and held before pasteurization. This process causes MFGM damage and LPL release and, additionally, reduces fat globule size and increases total fat globule surface area, providing a larger lipid-serum interface for lipase activity [38]. This is consistent with values reported by other investigators Efthymiou [25, 27, 39–41], while for 60-day-old Feta cheese made with different rennet types, higher values have been reported [26, 42, 43]. Butyric, caproic, caprylic, and capric acid levels were low in all cheeses (Table 2), indicating low lipolytic activity stemming exclusively from the starter microorganisms. Besides, butyric acid ($C_{4:0}$) is also an essential component of Feta cheese, contributing significantly to its flavor and piquant taste. Among all saturated fatty acids, Palmitic acid was the most dominant FFA, while oleic acid was the dominant one among all of the unsaturated fatty acids found in all cheeses after the same ripening periods. This finding is in agreement with the result of a number of investigators without regard to the level of acetic acid; these investigators found that acetic acid and palmitic acid were the main FFAs in Feta cheese salted only with NaCl and aged for 40 and 120 days [27]. These results agree with those of investigators who reported that in Cheddar cheese, the substitution of more NaCl with KCl greatly increased lipolysis [44]. The mean concentrations of total volatile free fatty acids (TVFFA) for UF white cheese are shown in Table 2. No significant differences ($P < 0.05$) were detected among volatile FFAs in all treatments and after the same ripening time. This finding is in agreement with Katsiari et al.'s results [24], which reported no significant differences ($P < 0.05$) between control and experimental Feta cheeses in TVFFA after the same ripening periods of 40 and 120 days.

### 3.3. Chemical Composition of Cheese.

Table 3 shows that Iranian UF Feta-type cheese salted with various mixtures of NaCl and KCl did not greatly affect the moisture, dry matter, fat, TN/dry matter, and WSN contents of cheeses; however, the moisture after all treatments slightly decreased during storage. This could be due to the exudation of a slight quantity of cheese serum. The present data in Table 3 indicate that the use of KCl instead of NaCl for salting of Iranian Feta-type cheese did not significantly influence the TN/dry matter and WSN levels of cheese throughout the storage period. This is in agreement with the results obtained with Cheddar cheese by Rasmussen and Barbano [45]. The increases in WSN of the cheeses throughout the storage period could be attributed to the microbial and enzyme activities. The rate of protein breakdown at 4 °C increased during storage period. Generally, these N fractions increased throughout aging in all cheeses. This trend in the change of N fractions was similar to that reported by Katsiari et al. [23] for Kefalograviera cheese made by partial replacement of NaCl with KCl. The levels of the N fractions in the control and experimental cheeses were similar ($P < 0.05$) at all sampling ages (Table 3). These results agree with those of other workers [18, 23] for other cheese varieties. Fitzgerald and Buckley also found no significant differences in the WSN levels between control Cheddar cheese salted with NaCl and experimental cheeses salted with equivalent amounts of KCl or 1 : 1 mixture of NaCl/KCl [44].

### 3.4. Textural Properties of Cheese.

The results of the texture analysis after 30 days of ripening are shown in Figure 2. The evaluation of texture was based on a force-to-fracture test. The cheeses salted with the NaCl/KCl mixtures generally were more fracturable [40]. The evaluation of force to fracture showed that there were significant differences ($P < 0.05$) between treatment (d) as a control cheese and treatments (b) and (c). However, there were no significant

Table 3: Chemical composition** of Iranian UF* cheese** treated by KCl and NaCl during 30 days of storage period.

| Age at analysis (day) | Treatments | Moisture (%) | Dry matter (%) | Fat (%) | †TN%/dry matter | ‡WSN (%) |
|---|---|---|---|---|---|---|
| 1 | (a) | 64.02 ± 0.1[a] | 35.98 ± 0.02[a] | 15.00 ± 0.25[a] | 5.55 ± 0.01[a] | 0.28 ± 0.01[a] |
| | (b) | 64.15 ± 0.15[a] | 35.85 ± 0.15[a] | 15.25 ± 0.25[a] | 5.54 ± 0.03[a] | 0.28 ± 0.01[a] |
| | (c) | 64.00 ± 0.15[a] | 36.00 ± 0.15[a] | 15.25 ± 0.25[a] | 5.58 ± 0.02[a] | 0.28 ± 0.01[a] |
| | (d) | 64.03 ± 0.1[a] | 35.97 ± 0.03[a] | 15.25 ± 0.25[a] | 5.57 ± 0.07[a] | 0.28 ± 0.01[a] |
| 15 | (a) | 63.72 ± 0.3[a] | 36.28 ± 0.7[a] | 15.17 ± 0.08[a] | 5.54 ± 0.07[a] | 0.29 ± 0.01[a] |
| | (b) | 63.83 ± 0.32[a] | 36.17 ± 0.23[a] | 15.25 ± 0.25[a] | 5.57 ± 0.03[a] | 0.29 ± 0.01[a] |
| | (c) | 63.80 ± 0.17[a] | 36.20 ± 0.2[a] | 15.50 ± 0.25[a] | 5.56 ± 0.1[a] | 0.29 ± 0.01[a] |
| | (d) | 63.77 ± 0.26[a] | 36.23 ± 0.2[a] | 15.25 ± 0.25[a] | 5.64 ± 0.01[a] | 0.29 ± 0.01[a] |
| 30 | (a) | 63.31 ± 0.41[a] | 36.69 ± 0.41[a] | 15.57 ± 0.32[a] | 5.61 ± 0.07[a] | 0.29 ± 0.01[a] |
| | (b) | 63.20 ± 0.63[a] | 36.80 ± 0.63[a] | 15.00 ± 0.25[a] | 5.62 ± 0.05[a] | 0.29 ± 0.01[a] |
| | (c) | 63.15 ± 0.65[a] | 36.85 ± 0.65[a] | 15.25 ± 0.25[a] | 5.66 ± 0.1[a] | 0.29 ± 0.01[a] |
| | (d) | 63.45 ± 0.32[a] | 36.55 ± 0.32[a] | 15.25 ± 0.25[a] | 5.63 ± 0.01[a] | 0.29 ± 0.01[a] |

*means in each column with the same superscript did not differ significantly ($P > 0.05$). **means of two trials. Means ± standard error. †TN : total nitrogen. ‡WSN : water-soluble nitrogen. **(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00%, KCl, and (d) 0.75% NaCl+2.25% KCl.
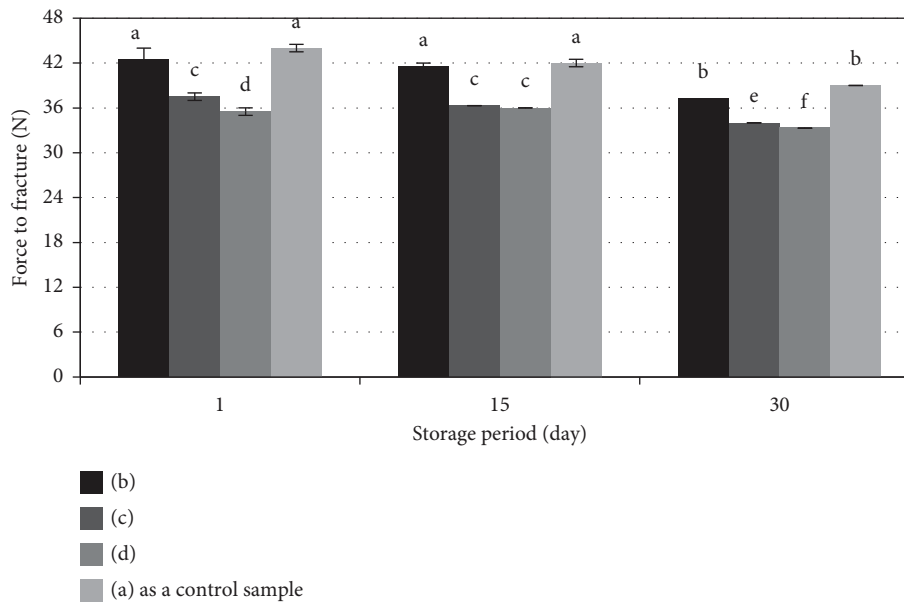


Figure 2: Changes in force to fracture (N) during storage of Iranian UF cheese* treated by KCl and NaCl during 30 days of storage period. * (a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl.

differences ($P < 0.05$) between treatments (d) and (a). This finding is not in accordance with the result of Katsiari et al, who reported no significant differences in force to fracture in Feta cheese salted with a 3 : 1 NaCl/KCl mixture and control cheese [40]. However, this study's finding is consistent with the results of other investigators who reported no significant differences in physical characteristics (firmness, shortness, and hardness) of Cheddar cheese salted with KCl or a 1 : 1 NaCl/KCl mixture and the control cheese [44]. During the aging of many types of cheese, the protein network changes from a granular structure to a homogeneous mass and softening occurs. These changes are probably due to the proteolysis of $\alpha_s^{-1}$ casein, mainly by residual coagulant [46]. Low sodium tends to produce weak, soft, and pasty cheeses, whereas cheese with higher salt concentration tends to be short and brittle with dry and hard body [47]. Similar results have been reported for Gaziantep [48] and Feta cheeses [49].

This may be due to the influence of NaCl on composition, biochemical changes during ripening, casein hydration, and impact on pH of cheeses [47, 50]. Rulikowska et al. [51] tested the textural properties of Cheddar cheeses prepared by varying the rate of salt addition over a period of 224 days. The authors observed an inverse relationship of salt content with firmness, fracture stress, and fracture strain. The decrease in hydration with the reduction of NaCl along with increased proteolysis may be associated with the decrease in textural attributes. In another study, Euston et al. [52] planned to evaluate the textural profile of low-sodium Cheddar cheeses by keeping the compositional parameters constant and reported that casein hydration is primarily responsible for the viscoelastic characteristics of cheeses. Ganesan et al. [53] investigated the impact of NaCl content (0.7%, 1.0%, 1.25%, 1.35%, and 1.8%) on textural properties (hardness, resilience, cohesiveness, chewiness, adhesiveness,
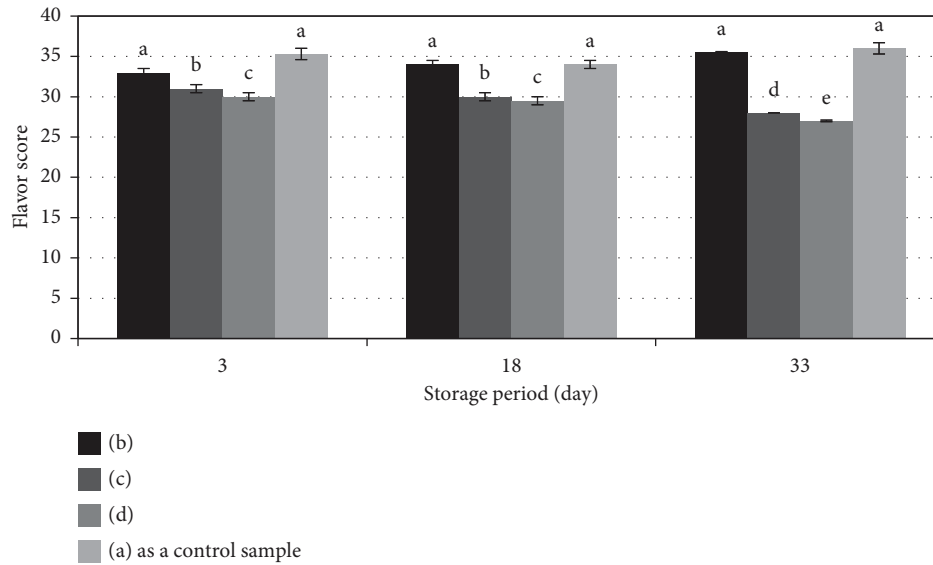
FIGURE 3: Changes in flavor score of Iranian UF cheese* treated by KCl and NaCl during 33 days of storage period. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl.

and springiness) of Cheddar cheese at room temperature (22 °C) after 3 and 6 months of storage. The authors noted no effect of salt level on resilience, cohesiveness, and hardness of cheeses toward the end of 90 days of ripening.

### 3.5. Sensory Evaluations

*3.5.1. Flavor Score.* The results of the taste panel's assessment of cheese flavor during 33 days of storage are shown in Figure 3. The mean scores for flavor of cheeses salted with mixtures of NaCl/KCl were significantly different ($P < 0.05$) from those of the control cheese at all sampling ages. The control sample had the highest scores in comparison to other treatments and the lowest flavor scores were for treatment (c); however, the flavor scores for treatment (a) were similar to those for treatment (d) (control). The perceived taste of salt depends on the nature of its anion and cation. Whenever the molecular weight of the anion and cation increases, the taste tends toward bitterness. The main reason for different flavor scores was attributed to different concentrations of KCl in combination with NaCl. On the one hand, $K^+$ has a substantially bitter taste; on the other hand, whenever the amount of $K^+$ increased over that of $Na^+$, the bitter taste was more evident. Treatment (d) had no bitter taste, and thus was awarded the highest score. Furthermore, the flavor of cheeses was more evident after longer ripening, and the panelists may simply have distinguished differences among various treatments with respect to the ripening period. As discussed above, as the concentration of KCl increased, the cheese gradually become less acceptable. The cheese salted at a ratio of 1:1 NaCl/KCl received lower scores for flavor than the control cheese, and panelists reported that it had a bitter, metallic flavor. Lower flavor scores for cheese salted with the ratio 1:1 NaCl/KCl than for the control samples were due to the slightly bitter, metallic aftertaste typical of KCl, especially after 18 and 33 days of storage. This result does not agree

with that stated by other investigators, who had found that "the mean scores for the flavor of cheeses salted with the mixture of NaCl and KCl were not significantly different ($P < 0.05$) from those of the control cheese at all sampling ages" [40]. But these results did agree with other researchers who had concluded that control cheeses received numerically higher flavor scores than NaCl/KCl salted cheeses. This result is also in agreement with those of investigators examining Feta-type cheese [40]. Both studies showed that cheeses containing 1% NaCl+1% KCl had similar flavor scores to control cheeses. Other studies showed that a reduction of Na from 25 to 50% in surfaced-salted cheese crackers had no adverse influence on sensory attributes [54].

*3.5.2. Body and Texture Scores.* Organoleptic scores from UF white cheese obtained from all treatments are shown in Figure 4. The assessment of the body and texture of UF white cheese showed that the effect of treatment was significant ($P < 0.05$). The control cheese at all sampling ages received the highest score for body and texture characteristics, and treatment (c), which had the highest content of potassium chloride, received the lowest score. Treatment (a) had a higher score than treatments (b) and (c). The score for body and texture for treatment (a) was similar to that for the control cheese. Overall, treatments with higher potassium chloride content were more fracturable and shortened and less hard [44].

*3.6. Aroma Compounds.* The flavor of cheese originates from microbial, enzymatic, and chemical transformations. The breakdown of milk proteins, fat, lactose, and citrate during ripening gives rise to a series of volatile and nonvolatile compounds which may contribute to the cheese flavor. Several degradation types occur simultaneously and the ultimate result will be a very wide range of compounds. The
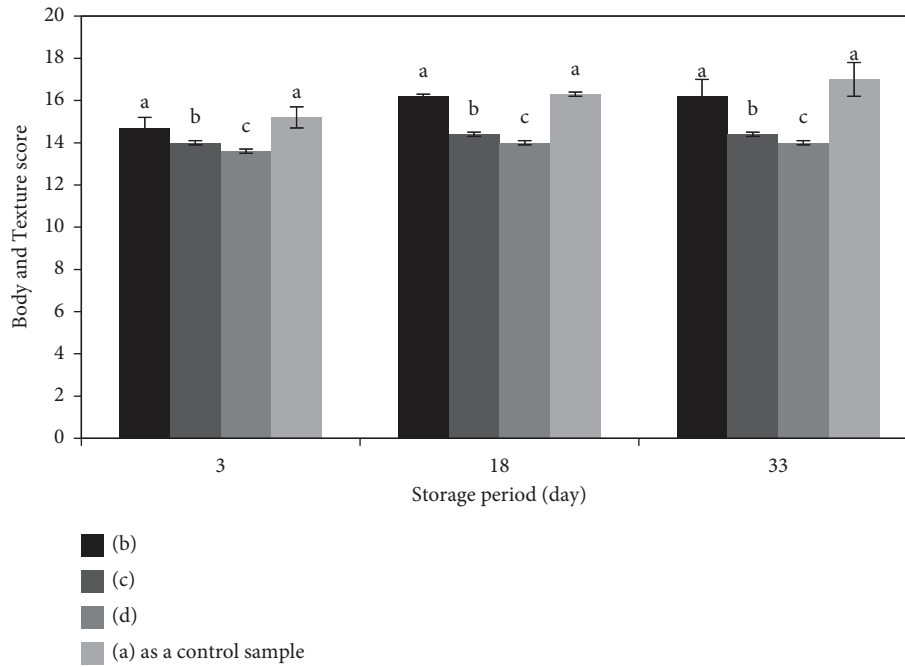
FIGURE 4: Changes in body and texture score of Iranian UF cheese* treated by KCl and NaCl during 33 days of storage period. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl.
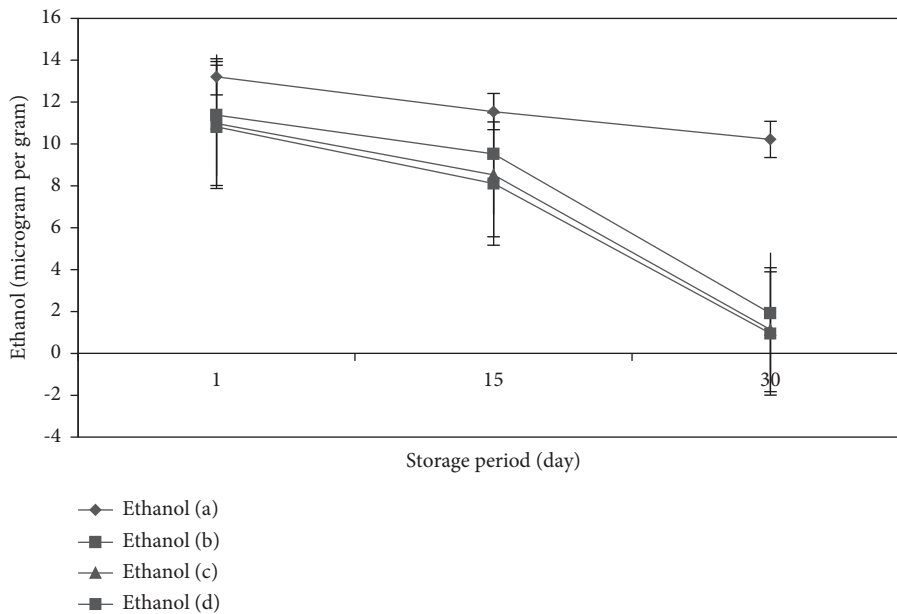


FIGURE 5: Changes in acetoin** ($\mu$g/g) during storage of Iranian UF cheese* treated by KCl and NaCl during 30 days of storage period. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl. (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl. **means at the same age differed significantly ($P > 0.05$).

factual contribution of them to the flavor of cheese is largely unknown. Proteolysis in cheese during ripening plays an important role in the development of texture. However, it also contributes to the taste of cheese by the production of peptides and free amino acids. Large peptides do not contribute directly to the cheese taste but can be hydrolyzed to shorter peptides that may be bitter [55]. Free amino acids are the final products of proteolysis. Very extensive proteolysis

occurs in blue-mold cheeses [56]. The identification of the individual compounds in the sample is difficult, owing to their low concentrations in cheese and the relatively high concentrations of other compounds. The identification was carried out by GC and confirmed by comparing the retention times with those of standard substances. Fatty acids are important components of the flavor of many cheese types. They may originate from lipolysis, a lower proportion
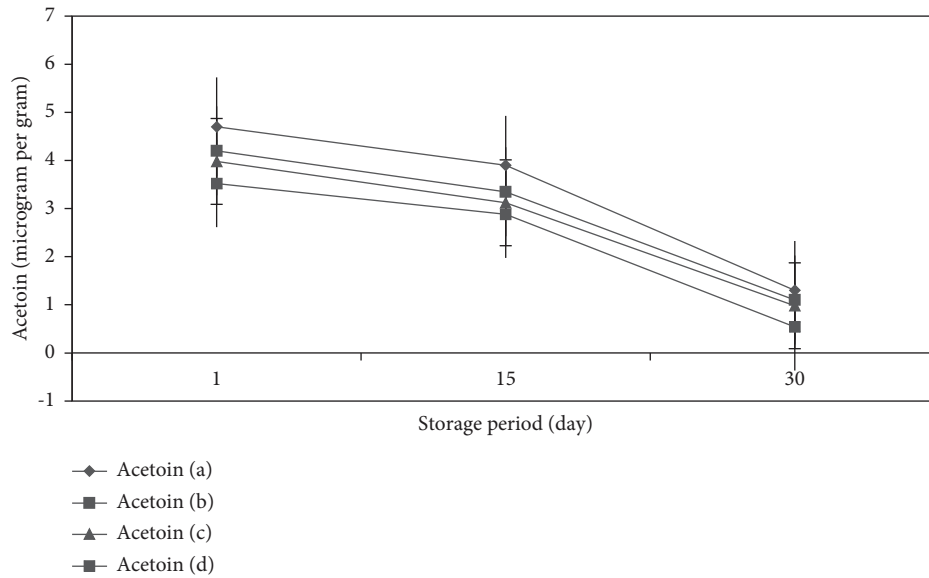
FIGURE 6: Changes in ethanol** ($\mu$g/g) during storage of Iranian UF cheese* treated by KCl and NaCl during 30 days of storage period. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl. **means at the same age differed significantly ($P > 0.05$).
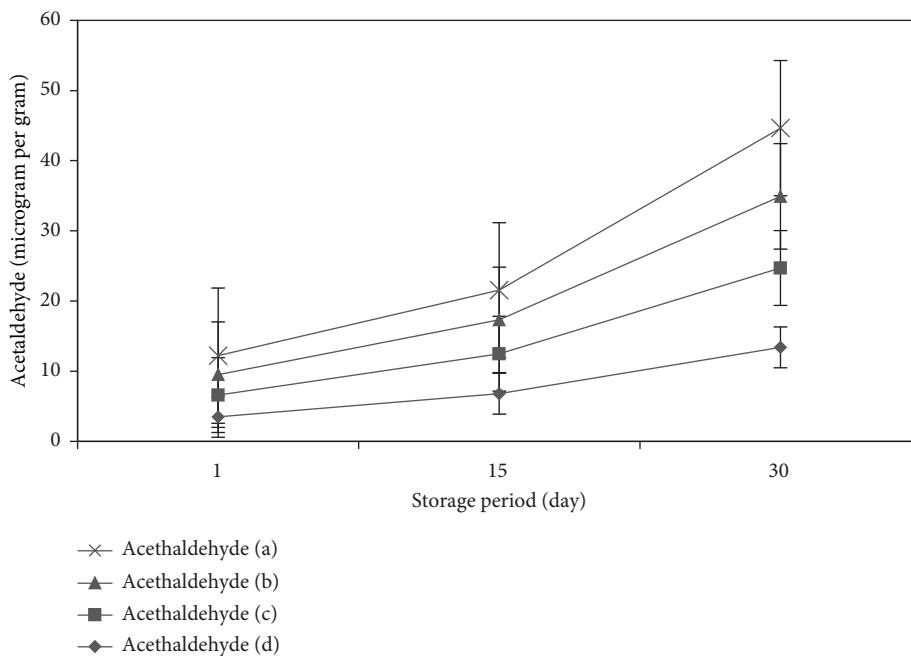


FIGURE 7: Changes in acetaldehyde** ($\mu$g/g) during storage of Iranian UF cheese* treated by KCl and NaCl during 30 days of storage period. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl. **means at the same age differed significantly (($P < 0.05$).

of short-chain FA originate from the degradation of lactose and amino acids, and they can also be derived from ketones, esters, and aldehydes by oxidation [57]. Long-chain FA (>12 carbon atoms) play a minor role in the flavor owing to their relatively high perception thresholds. The amount of ethanol in all treatments decreased during the 30 days of storage (Figure 5). However, the intensity of reduction was greater in treatments (a), (b), and (d) (Figure 6). Ethanol comes from

lactose fermentation. It has a limited role in the cheese aroma despite its high levels, but it contributes to the formation of esters [58]. The amount of acetoin in all treatments decreased during the 30 days of storage (Figure 6). Ketones are common constituents of most dairy products, which may be reduced to secondary alcohols [58]. Methyl ketones are derived from FA by $\beta$-oxidation or from $\beta$-ketoacids and are primarily known for their contribution
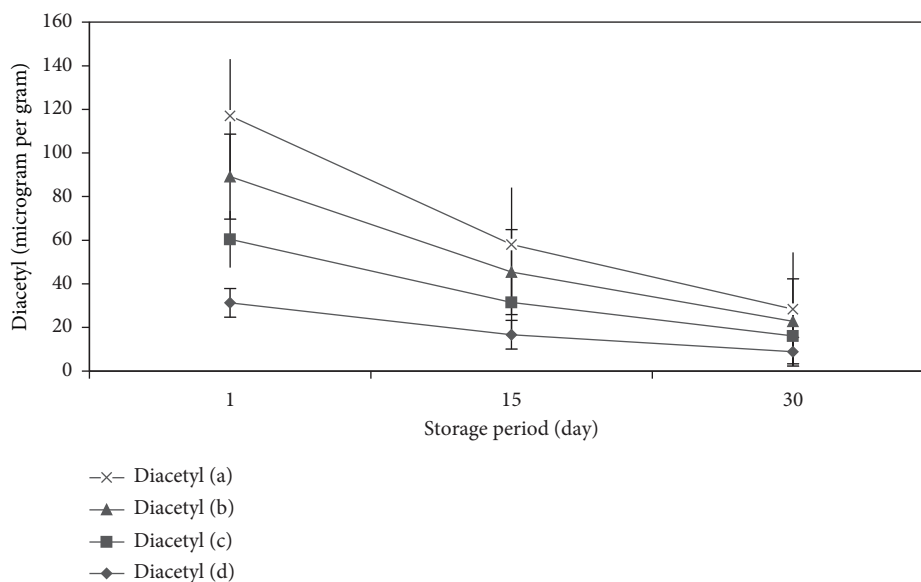
FIGURE 8: Changes in diacetyl** ($\mu$g/g) during storage of Iranian UF cheese* treated by KCl and NaCl during 30 days of storage period. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl. **means at the same age differed significantly ($P < 0.05$).

to the aroma of mold cheeses. They have typical odors (fruity, floral, mushroom, or musty notes) and low perception thresholds [59]. The amount of acetaldehyde in all treatments increased during the 30-day storage period (Figure 7). Primary alcohols are formed by the proper aldehyde reduction. They impart a fruity, nutty note to the cheese flavor, and in certain cheeses, high levels of them can cause flavor defects. Secondary alcohols are formed by enzymatic reduction of the corresponding methyl ketones [55]. They have similar but heavier flavor notes than methyl ketones. The amount of diacetyl in all treatments decreased during the 30-day storage period (Figure 8). One of the most important diketones is diacetyl (butan-2,3-dione) with a sweet buttery and vanilla aroma. It is formed through lactose and citrate metabolism and its production is mainly due to the activity of lactic acid bacteria. It can be reduced to acetoin (3-hydroxybutan-2-one) with a buttery aroma and the latter can be further reduced to butane-2,3-diol, which does not have a flavor impact [57]. Diacetyl was identified as very important to blue cheese, in which acetoin was also detected. As mentioned previously, in mold cheese, methyl ketones are the most abundant aroma compounds, the major ones being heptan-2-one and nonan-2-one [58–60].

## 4. Conclusions

The findings of this study indicated that a reduction of sodium of up to 50% in UF white cheese, attained by the partial replacement of NaCl with KCl, did not affect lipolysis significantly, as determined by the ADV method and gas chromatography during the ripening period of cheese. Iranian UF Feta-type cheese salted with various mixtures of NaCl and KCl did not greatly affect the moisture, dry matter, fat, TN/dry matter, and WSN contents of cheeses. The evaluation of force to fracture showed that there were

significant differences ($P < 0.05$) between treatment (d) as a control cheese and treatments (b) and (c). However, there were no significant differences ($P > 0.05$) between treatments (d) and (a). Sensory evaluations of flavor and body and texture showed that, as the concentration of KCl increased, the cheese gradually became less acceptable and treatments with higher potassium chloride content were more fracturable and shortened and less hard. The results of the aroma evaluation of cheese samples showed that unlike acetaldehyde, the amounts of ethanol, acetoin, and diacetyl decreased significantly ($P < 0.05$) during the storage period.

[a]means in each row and at the same age without a superscript did not differ significantly ($P > 0.05$); means in each row and at the same age denoted with a superscript differed significantly ($P > 0.05$). [b] means of two trials. Means ± standard error. [c] Total volatile free fatty acids. *(a) 3% NaCl (control), (b) 1.50% NaCl+1.50% KCl, (c) 1.00% NaCl+2.00% KCl, and (d) 0.75% NaCl+2.25% KCl.

## Data Availability

The data that support the findings of this 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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