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Changes in the rheological properties of Iranian UF-Feta cheese during ripening

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ABSTRACT

The frequency sweep test was used to evaluate storage modulus (G′), loss modulus (G″) and loss tangent [tan (δ)] of Iranian UF-Feta cheese during ripening period (3, 20, 40 and 60 days). With development of ripening, storage and loss moduli increased at varying rates. The rate of increase in G′ was greater than that in G″ resulting in a reduction in tan (δ). That is, storage modulus was dominant to loss modulus and as a result the elasticity nature was greater than the viscous nature of cheese samples. Due to the disruption of fat globules and proteolysis, protein matrix rearranged and formed a more compact texture containing aggregates of casein. Ripening did not influence the pH level and also the concentrations of dry matter, fat, salt, and total nitrogen in dry matter. However, water soluble nitrogen increased significantly ($P < 0.05$).

1. Introduction

The chemical and physical changes occurring during ripening, cause the body of the freshly made cheese to lose its firmness, toughness and curdy texture (Tunick, 2000). Cheese ripening is a complex process involving many physicochemical changes such as a change in pH, a progressive breakdown of the proteins to smaller polypeptides and the gradual accumulation of amino acids (Fox, Law, McSweeney, & Wallace, 1993). Cheese texture may also vary with a change in the physical state of the fats that are already present in the cheese (Dufour, Mazerolles, Devaux, Duboz, Duployer, & Mouhous Riou, 2000; Watkinson et al., 1997). There are varieties of soft cheeses due to the differences in factors such as the composition of the curd (levels of fat, protein and water), pH, curd structure, salt content, ripening conditions and degree and mode of fat and protein breakdown during ripening (Gripon, 1992; Karahadian & Lindsay, 1987). Rheological characterization of cheese is important as a means of determining body and texture characteristics and also for examining how these parameters are affected by composition, processing techniques and storage conditions (Konstance & Holsinger, 1992).

Instrumental measurements of food texture are in turn based on the rheological properties that food exposes (Steffe, 1996). Dynamic low-amplitude strain testing offers quick analysis with minimal chemical or physical changes (Tunick, 2000). Small-strain dynamic rheological methods are useful to characterize cheese varieties and also to differentiate them from each other by defining both the elastic and viscous natures of cheeses (Tunick, 2000). Such methods are implemented within the linear viscoelastic region of the material and therefore, are designed to be non-destructive to its basic structure (Gunasekaran & Ak, 2000). When performing tests within the linear viscoelastic region, the elastic and loss moduli will be dependent on the time and magnitude of the stress/strain applied (Tunick, 2000). There are several published articles in the literature reporting on the rheological, textural, and sensory characteristics of Feta cheese (Katsiari, 1997; Lalos, 1996; Pappas, 1996; Samal, Pearce, Bennet, & Dunlop, 1993; Sipahioglu, Alvarez, & Solano-Lopez, 1999; Wium, Gross, & Qvist, 1997; Wium & Qvist, 1997). However, only a few of them have reported on the dynamic properties of this kind of cheese (Wium & Qvist, 1998; Wium et al., 1997).

During ripening, several biochemical events (primarily proteolysis, glycolysis, and lipolysis) along with the slow solubilization of some of the residual colloidal calcium phosphate (CCP) change curd from a rubbery bland product to a mature cheese with a characteristic texture, flavour, and aroma (Fox, Lucey, & Cogan, 1990). Proteolysis is the most important biochemical reaction in most cheese varieties during ripening (Fox et al., 1993).

One of the main steps during cheese manufacture is whey removal, which results in the concentration of the major constituents (Mistry & Maubios, 1993). Ultrafiltration (UF) offers an alternative way of concentrating milk before the formation and handling of the curd (Mistry & Maubios, 1993). UF has been successfully applied in Feta cheesemaking (Tamime & Kirkegaard, 1991). Due to the high concentration of whey proteins in UF cheeses, they ripen more slowly than the traditional ones (Mistry & Maubios, 1993).

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Iranian UF-Feta cheese made from bovine milk is manufactured in modern dairy plants from ultrafiltered and pasteurized milk with mesophilic starter cultures and commercial microbial rennet. The main characteristics of this cheese type are a minimum of 34% (w/w) total solids, a protein content of 11%, fat content of 15%, 27 ° brix, maximum acidity of 42 and a pH of 6.20–6.65. High contents of whey proteins in UF cheeses result in some problems for both the ripening and textural characteristics of these types of cheeses. To rectify these problems, physicochemical and structural/rheological properties of the cheeses need to be determined. The published reports on the rheology and texture of Feta cheeses mainly concern those made traditionally without any attention to the modern technology of ultrafiltration. Therefore, the purpose of this study was to evaluate the rheological changes of Iranian UF-Feta cheese made by UF modern technology during the ripening period.

2. Materials and methods

2.1. Materials

Starter cultures (DM-230 and Y-502) with combination of *Lactococcus. lactis subsp. cremoris* and *L. lactis subsp. lactis* obtained from Danisco Deutschland GmbH (Alemanha, Germany) and rennet (Fromase® 2200 TL granulate, ⩾2200 IMCU g^{-1}) as microbial coagulant from *Rhizomucor miehei* supplied by DSM Food Specialities (Seclin, France). Raw cow's milk, equipment and filtration moduli were provided by Hamedan Pegah dairy complex (Hamedan, Iran).

2.2. Methods

2.2.1. Cheesemaking

Experimental cheese samples were made in three replicates at "Hamedan Pegah" dairy plant (Hamedan, Iran) according to a UF cheesemaking method (Bylund, 1995) proposed by Tetra-Pak Processing Systems AB (Lund, Sweden) adapted (with some modifications) by Hesari, Ehsani, Khosroshahi, and McSweeney (2006) for the production of Iranian UF-Feta cheese. After the bactofugation, pasteurization (76 °C for 15 s), ultrafiltration and homogenization stages, the retentate with a volume concentration factor of 5.4 kg milk to 1.0 kg retentate entered the starter tank, where by adding the starter (1 g starter for 1000 kg Feta cheese), pH of milk reached to 6.2 level. Then, in the filler, rennet was mixed with water (2 g for 100 kg retentate) and added to each cheese container. Coagulation tunnel, which was set at 37 °C for 30 min, allowed the retentate to be converted to a pre-cheese mixture before entering to other stages of the process. In the sealing machine, 4% (w/w) salt was added onto the parchment paper on the top of cheese and then by using aluminum foil, the container was sealed. In the pre-ripening stage (37 °C), after dropping the cheese pH to 4.80, cheese samples were transferred to a cold-room (9 ± 1 °C) for ripening purposes (3–60 days), after which chemical, rheological and microstructural analysis were performed.

2.2.2. Physicochemical analysis

The major compositional factors affecting cheese properties are the pH, moisture, fat and salt content (Gunasekaran & Ak, 2003). A Knick 766 calimatic pH-meter (Niels Bohrweg, Utrecht, The Netherlands) was used for measuring the pH of cheese samples. Cheese samples were analyzed in triplicate for moisture by heating to a constant weight using a Sartorius moisture analyzer (Sartorius Ltd., Epsom, UK) and fat according to the BSI (British Standard Institute, 1995) method. Salt concentration was determined according to a procedure described by Kirk and Sawyer (1991). As one of the indices for the progress in the proteolysis reaction during the ripening period, total nitrogen (TN) was determined applying the Kjeldahl method (IDF, 1993). Water soluble nitrogen (WSN) as the second index was measured by using a method described by Alizadeh, Hamedi, and Khosroshahi (2006), where 20 g of cheese samples was homogenized in water (100 mL) using a stomacher for 5 min and the suspension was maintained at 40 °C for 1 h. After incubation, the insoluble solids were separated by centrifugation (3000 rpm) at 4 °C for 30 min. The supernatant was then filtered through glass wool and the nitrogen content in the filtrate was determined according to Kjeldahl method.

2.2.3. Dynamic rheological measurements

A Universal Dynamic Spectrometer, Paar Physica UDS 200, rheometer (Physica Messtechnik GmbH, Stuttgart, Germany) was used to determine small-amplitude oscillatory shear measurements following (with some modifications) a method adapted by Madadlou, Khosrowshahi, Mousavi, Emamdjome, and Zargaran (2007) to measure frequency sweep test. The primary viscoelastic terms (the storage, G', and loss moduli, G'') along with phase angle tangent, tan (δ), were determined (Tunick, 2000). The measurement system consisted of two parallel plates with a diameter of 25 and a gap size of 1.0 mm (sample thickness). Samples were cut from the center of cheese blocks at 9 ± 1 °C and were immediately placed in plastic bags, sealed and equilibrated at room temperature (25 ± 1 °C) for at least 5 h. A small piece of cheese was then placed on the lower plate and then the upper plate was slowly moved down until the pre-set gap size was reached. The extra cheese parts were trimmed off carefully with a razor blade and the sample was let relax for 15 min in the rheometer to allow stresses induced during sample handling to relax. The linear viscoelastic range was obtained by performing a strain sweep test at 0.1 Hz frequency as the percentage of strain values varied from 0.01–2.00%. A strain in the linear region was then selected (0.02) and a frequency sweep test was performed from 0.1 to 100 Hz. Means of two measurements for three replicates of cheese samples were reported.

2.2.4. Microstructure

Cheese samples (in triplicate) were prepared for SEM (scanning electron microscopy) analysis after 3, 20, 40 and 60 days of ripening following a method described by Drake, Herrett, Boylston, and Swanson (1996) with some modifications suggested by Madadlou, Khosrowshahi, Mousavi, and Emamdjome (2006), Madadlou et al. (2007) and applied for the evaluation of textural characteristics of low-fat Iranian white cheese. Using a sharp razor, cheese blocks were cut in 5–6 mm^3 cubes and immersed in 2.5% (w/w) gluteraldehyde fixative (Merck, Darmstadt, Germany) for 3 h. To avoid possible changes in the fat microstructure or the likely loss of fat from cheese matrix, the washing and dehydration stages used by Drake et al. (1996) and Madadlou et al. (2006), Madadlou et al. (2007) were not applied in this study. Samples were refrigerated until used for SEM analysis. During SEM analysis, samples were freeze-fractured in liquid nitrogen (Sipahioglu et al., 1999) to approximately 1-mm pieces and these pieces were then mounted on aluminum stubs with silver paint, dried to critical point and coated with gold for 300 s in a sputter-coater (Type SCD 005, Bal-Tec Inc., Balzers, Switzerland). Samples were viewed with a scanning electron microscope (XL Series, model XL30, Philips, Eindhoven, The Netherlands) operated at 20.0 kV. Images were recorded at 250, 500, 1000, 2500 and 5000 magnification levels.

2.2.5. Statistical analysis

Experimental data (dry matter, fat, salt, total nitrogen/dry matter (TN/DM), water soluble nitrogen/total nitrogen (WSN/TN) and pH) were statistically analyzed using SAS statistical software (Version 8.2, SAS institute Inc., Cary, NC). A multifactor analysis of variance using the LSD test ($P < 0.05$) was used to examine the influence of ripening period (3, 20, 40 and 60 days) on the physicochemical characteristics of cheese samples. All the charts were drawn using Microsoft Office Excel 2007.

3. Results and discussion

3.1. Physicochemical characteristics

Changes in dry matter, fat, salt, total nitrogen in dry matter (TN/DM) and water soluble nitrogen in total nitrogen (WSN/TN) concentration along with the changes in pH value of a series of UF-Feta cheeses throughout the ripening (3, 20, 40 and 60 days) are shown in Fig. 1. With an increase in the ripening period, the concentrations of dry matter, fat and salt and pH value did not indicate any significant changes. The pH value of a 3-day-old cheese and that of a 60-day-old cheese was similar (4.36–4.40). Such pH values are necessary for a mature Feta cheese to maintain its good quality during storage (Anifantakis, 1991). This can be related to the high permeability of lactose through the UF membrane (Tamime & Kirkegaard, 1991). As a consequence, the resultant lactic acid from residual lactose in UF-Feta cheese was low and therefore, no significant pH changes were observed during the ripening period. In addition, minerals bound to casein micelles result in an increase in buffering capacity of UF retentates and change the acidification kinetics of lactic acid bacteria as well as the final pH value (Mistry & Maubios, 1993). During cheese ripening, released amino acids raise pH value to a somewhat higher level (Waagner Nielsen, 1993). In high-pH cheeses (>5.2), absorption of water is very high but is limited at low-pH (Fox, Guinee, Cogan, & McSweeney, 2000). At low-pH (<5.2), colloidal calcium phosphate dissociates from casein micelles and progressive breakdown of submicelles into small aggregates of casein take place (Fox et al., 2000). When pH reaches to the isoelectric pH of casein (4.6), protein matrix becomes compact and short. According to Lawrence, Creamer, and Gilles (1987), at pH < 4.8 (as is the case in the current study), the casein aggregates are found in the form of non-linear strands. In our study, the TN/DM ratio, which was measured at 6.2 ± 0.1% on day 3, did not change significantly throughout the ripening period. However, changes in WSN/TN (5.2 ± 0.1–6.8 ± 0.2) were significant ($P < 0.05$, Fig. 1). Compared to the data reported by Kandarakis, Moatsou, Georgala, Kaminarides, and Anifantakis (2001) and Georgala et al. (2005) for traditional Feta cheese, the level of proteolysis for UF-Feta cheeses obtained in this study was lower. This finding is also in good agreement with the data reported by Hesari et al. (2006). As verified by Rao and Renner (1989), β-lactoglobulin as a whey protein inhibits plasmin activity in UF cheeses and blocks the conversion of β-casein to γ-casein and proteose peptone. In this way, proteolysis is retarded and some textural and sensorial defects take place. The fat, salt and dry matter contents and pH of matured cheeses met the specifications for "first quality" Feta cheese as described by FCC (Food Chemicals Codex) (Codex Alimentarious, 2003) and also those of Iranian standards for UF-Feta cheese.

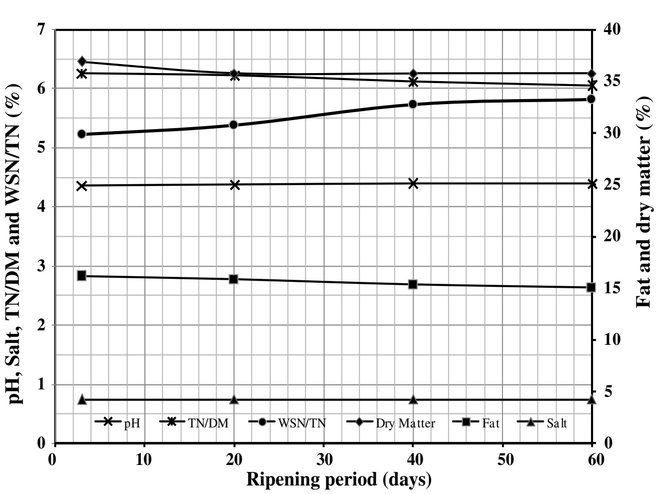

Fig. 1. Changes in the concentration of dry matter and fat (secondary axis), salt, pH, TN/DM and WSN/TN (primary axis) of Iranian UF-Feta cheese during ripening.

3.2. Rheology

Frequency sweep test was carried out to determine viscoelastic behavior of UF-Feta cheese. The frequency sweep is probably the most common mode of oscillatory test (Tunick, 2000). Because, it shows how the viscous and elastic behaviors of the materials change with the rate of strain/stress application (Gunasekaran & Ak, 2003). Changes in the rheological properties of Iranian UF-Feta cheese during the ripening based on its storage modulus, loss modulus and loss tangent are shown in Figs. 2–4, respectively.

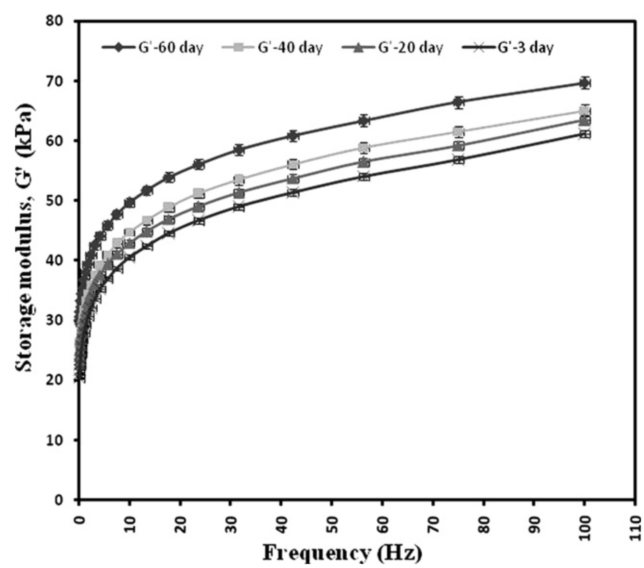

Fig. 2. Storage modulus (G′) of cheese samples during ripening period.

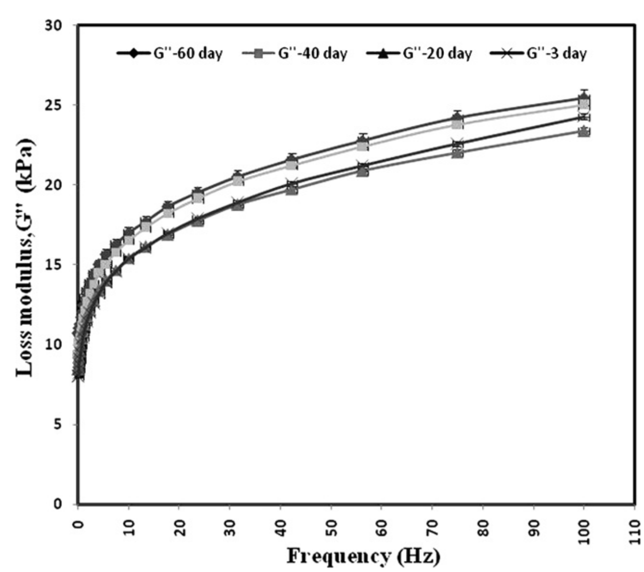

Fig. 3. Loss modulus (G″) of cheese samples during ripening period.

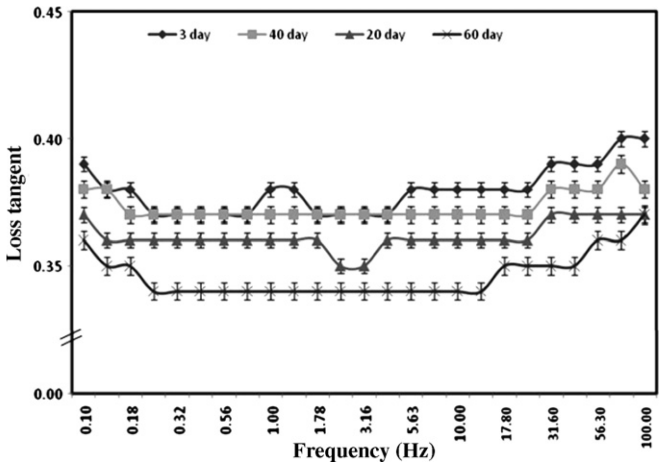

Fig. 4. Loss tangent [tan (δ)] of cheese samples during ripening period.

3.2.1. Storage and loss moduli

Figs. 2 and 3 show the changes in the storage and loss moduli during the ripening of Iranian UF-Feta cheese as a function of oscillation frequency. It can be seen that the moduli significantly increased as the ripening continued. With an increase in the ripening period (3, 20, 40 and 60 days), storage and loss moduli increased at different rates. When $G' > G''$ (gel character), the elastic behavior dominates over the viscous behavior and when $G'' > G'$ (liquid character), the viscous behavior dominates over the elastic behavior (Steffe, 1996). In the latter case, the sample shows the character of a liquid in the linear viscoelastic range (Karoui & Dufour, 2003). Three-day-old cheeses indicated the lowest storage and loss moduli throughout the frequency range studied. G' modulus of cheese samples in the initial stages of ripening (day 3) increased from 20.4 to 61.2 kPa at the frequency range of 0.1–100 Hz. Such changes were within 29.5–69.8 kPa (at the same frequency range) for the 60-day-old cheese samples. In a similar way, at the same frequency range (0.1–100 Hz), G'' modulus increased from 8.0 to 20.4 kPa for the 3-day-old cheese samples and from 10.7 to 25.5 kPa for the 60-day-old samples.

3.2.2. Loss tangent

Loss tangent was determined during the 3–60 days of ripening period (Fig 4). With an increase in the ripening period, tan (δ) decreased from 0.39 to 0.40 kPa at 0.1–100 Hz frequency range for the 3-day-old cheese to 0.36–0.37 kPa at the same frequency range for the 60-day-old cheese.

3.2.3. Overall results

Loss tangent was lower at lower frequencies showing that effective bonds (related to casein matrix) in UF-Feta cheese had a more elastic character at longer stress time. Wium et al. (1997) showed that tan (δ) decreased with increasing the frequency of oscillation from 0.01 to 10 Hz for three kinds of UF-Feta cheeses. They also suggested that the lower pH of Feta cheese (4.5–4.7) was probably the main reason for its dominant elastic character. pH has a major role on the viscoelastic properties of cheese (Tunick, 2000). In a sample of soft cheese, regions with the lower pH had higher G' and G'' values and also the softening of cheese was dependent to pH (Karoui & Dufour, 2003). It is well known that high-pH milk gels exhibit significantly higher tan (δ) values than do the low-pH milk gels (Tunick, 2000). However, a maximum in tan (δ) may appear at pH 5.2 (Roefs, 1990; Van Valiet & Kikkert, 1989).

According to Steffe (1996) the numerical values for tan (δ) are close to unity for dilute solutions, 0.2–0.3 for amorphous polymers, and near 0.01 for glassy crystalline polymers and gels. Madadlou et al. (2007) reported the storage modulus of 750–1300 kPa at 1–9 Hz for low-fat Iranian white cheese made with unhomogenized cream. However, higher concentrations of fat and whey proteins in Iranian UF-Feta cheese compared to the traditional Feta cheese (Madadlou, Khosrowshahi, Mousavi, & Emamdjome, 2006; Madadlou et al., 2007) lead to a decrease in the storage modulus. Ultrafiltration results in the full recovery of whey proteins (that is, 20% of the total proteins in cheese) in the cheese matrix leading to a softer cheese with less shear stress (Hinrichs, 2001). Also, due to the accumulation of all the caseins in the retentate, the gel strength is also increased accordingly (Hinrichs, 2001). Singh and Waungana (2001) showed that the addition of whey proteins into the UF cheeses obtained a loose, pasty and cracked texture. High water binding capacity of whey proteins in the UF cheeses result in a poor consistency/texture that will be rectified as the ripening is continued (Lopez & Dufour, 2001). In the current study, the quantity of tan (δ) for Iranian UF-Feta cheese was less than 1.0 indicating that the storage modulus was more dominant than the loss modulus. That is, the elasticity nature of the samples was higher than their viscous nature. With an increase in the ripening period, the elasticity modulus increased at higher rates than those of the viscous modulus. G' and tan (δ) are useful parameters to differentiate texture among UF-Feta cheeses (Gunasekaran & Ak, 2003). According to the results of Wium et al. (1997), the complex modulus of the three types of UF-Feta cheeses studied by them ranged from 40 to 173 kPa and the loss tangent varied from 0.17 to 0.25. Wium et al. (1997) were also able to distinguish textures of different Feta cheeses based on the complex modulus (G^*) and phase angle (δ) measured with strain sweep or frequency sweep tests. Tunick et al. (1990) examined the textural differences between Cheddar and Cheshire cheeses based on their dynamic rheological parameters [G', G'', and complex viscosity (η^*)]. Such a distinction was not possible by other analytical methods. Drake et al. (1996) reported good correlations between hardness with G' and springiness with G''.

As it was the case in this study, during the manufacture of Feta cheese, a relatively high amount of rennet is used (Fox et al., 2000), which is maintained in the cheese texture in the UF-Feta cheeses. From all the added rennet to the milk, about 6% is active in the cheese curd (Scott, Robinson, & Wilbey, 1998) resulting in a high aggregation rate and a coarse casein network, which is responsible for the firm gel of Feta cheese (Gunasekaran & Ak, 2003). In a young Feta cheese, this firming effect is more dominant than the softening effect of the proteolysis; however, as storage continues, the proteolytic effect increases to an extent that the cheese becomes fully soft and short (Wium, Kristiansen, & Qvist, 1998; Wium & Qvist, 1998). Hesari et al. (2006) studied the contribution of rennet and starter to the proteolysis of Iranian UF white cheese and reported that in cheeses made without rennet casein degradation was not noticeable. That suggests little contribution of indigenous proteinases to proteolysis during the ripening of this cheese (Hesari et al., 2006). Hesari et al. (2006) argued that rennet and starter directly or indirectly contributed to the production of free amino acids, where the omission of either of these agents strongly reduced the amounts of free fatty acids produced during the ripening. As indicated, WSN/TN% increased significantly during the ripening period indicating the breakdown of caseins due to proteolysis. This in turn leads to the reorganization and weakening of protein matrix and therefore in the release of fat globules from the casein matrix (Tunick et al., 1993). The proteolytic action of the starter culture also affects the rheological and textural properties of cheese through the slow (but progressive) breakdown of caseins during the storage (Lawrence et al., 1987). Scanning electron micrographs of UF-Feta cheese showed that the fat globules were disrupted during the ripening period (Fig. 5A–F) as was also shown by Lopez, Camier, and Gassi (2007) for Emmental cheese. During

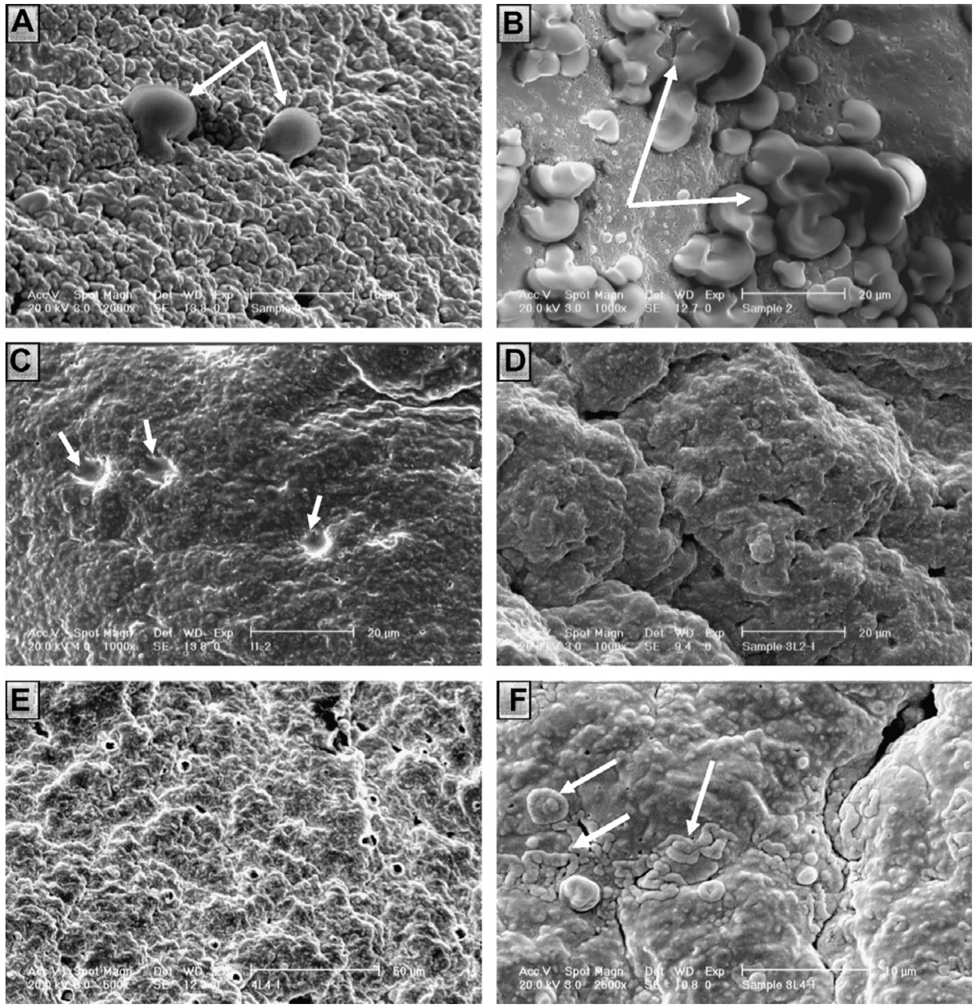

Fig. 5. SEM images of Iranian UF-Feta cheese texture during 3 (A, B), 20 (C), 40 (D) and 60 (E, F) days of ripening period. White arrows show: (A) individual fat globules, (B) aggregates of fat, (C) fingerprints of fat, (F) casein aggregates. Magnification level is below the images as a multiple of × (e.g., 1000×).

the initial periods of ripening (days 1–3) individual fat globules and aggregates of fat clearly were observed in cheese matrix (Fig. 5(A,B)). Fat disrupts the continuity of gel structure and increasing the number of fat globules in a process such as homogenization, therefore, increase the number of weak points in the cheese leading to a less elastic curd (Madadlou et al., 2007). On the other hand, fat present in the cheese curd acts as a plasticizer and inhibits the formation of cross-links between the casein chains (Gunasekaran & Ak, 2003). After 20 days of ripening, due to the lipolysis of cheese fat, fat globules were disrupted and only fingerprints of fat or free fat remained (Fig. 5C). This in turn resulted in the decreasing of the plasticizing effect of fat. Native milk lipase is sensitive to heat/pasteurization (Deeth, 2006). Lipolytic activity of lactic acid bacteria (LAB) has been previously reported (Collins, McSweeney, & Wilkinson, 2003). In the current study, LAB were used in the production of Iranian UF-Feta cheese as the starter. Due to the inactivation of milk lipase by pasteurization (76 °C for 15 s) and heating during the ultrafiltration (50 °C for 30 min), it seems that LAB are the main lipolytic agents maintained for the ripening stage of Iranian UF-Feta cheese. After 40 days of ripening, SEM images revealed a homogenous and compact texture (Fig. 5D) without any fat globule or its fingerprint. After 60 days, fat globules were completely disrupted and as a result of proteolysis, rearrangement of protein matrix occurred (Fig. 5E,F). As reported by Tunick (2000) for the cheese in general, low-pH (4.36–4.40) of UF-Feta cheese in this study also caused the breakdown of submicelles into non-linear strands of casein and finally to the aggregation of protein matrix. Casein aggregates were clearly observed after 60 days of ripening (Fig. 5F). Disruption of fat globules during the ripening of UF-Feta cheese resulted in a decrease in the weak points and an increase in the cross-links among the non-linear strands of casein. This in turn resulted in the elasticity of cheese and consequently a decrease in the tan (δ) (Fig. 4) as was the case for the studies of Wium and Qvist (1998); Wium et al. (1998).

4. Conclusions

With an increase in the ripening period, the storage and loss moduli increased while loss tangent decreased. Elasticity was greatly influenced by increasing the ripening period, which was due to the occurrence of proteolysis and lipolysis reactions during the ripening of the cheese. As a consequence, casein network started to rearrange and therefore new bonds (cross-links) were formed among the peptides due to the disruption of fat globules. Chemical properties (pH, dry matter, salt, fat and TN/DM contents) did not change significantly during the ripening period but WSN/TN% increased to some extent.

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