

The Effects of Storage Time on Physiochemical, Rheological, Micro-Structural and Sensory Properties of Feta Cheese Fortified with Fish and Olive Oils

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Abstract

Skim milk, milk fat, cumin powder, olive and fish oils used to produce fortified feta cheese with 8.4% mixed fat and its chemical, textural and sensory analyses compared with control or low only milk fat (again at 8.4%) feta cheese during 2 months cold storage. The measured hardness and sensorial scores of fortified cheese became very close (with no significant differences) to control samples after 30 days of storage due to the use of respectively whey protein concentrate and cumin seed powder. Additionally, the rheological properties (storage modulus, loss modulus and loss tangent) of fortified cheese reached to 85% of the parallel parameters in control sample throughout the 2 months cold storage. Although the peroxide and thiobarbituric acid values of fortified cheese increased to some extent, it did not exceed the standard levels and sensory panel did not detect any fishy flavour and unpleasant aroma even after 60 days storage. The unsaturated and saturated fatty acids of fortified cheese after 0, 30, and 60 days of storage, respectively were 120, 124 and 156% more and 27, 28 and 30% less than the similar values in control. Since the fortified samples of feta cheese had 30 mg or 10% of daily dose of EPA and DHA, it can be used as a rich source of omega 3 instead of normal feta cheese.

Keywords: Feta cheese; Rheological properties; Microstructure; Fortification

Introduction

The popular Iranian UF-Feta cheese is manufactured from ultra-filtered and pasteurized bovine milk mixed with mesophilic starter cultures and commercial microbial rennet. The minimum of 35% (w/w) total solids content (16% protein+17%fat+2% carbohydrate and minerals) and maximum pH of 5.2 are the main characteristics of standard feta cheese [1]. Although dairy products are good sources of bioavailable calcium, their milk fat contains about 70% saturated fatty acids (mainly myristic and palmitic) and has a high potential to raise low density lipoprotein (LDL) cholesterol and increase the risk of cardiovascular disease [2]. Even though lipids and essential fatty acids are important sources of energy and vital components of a balanced diet [3], every 1% increase or decrease in the intake of total calories obtained from saturated fatty acids, raise or reduce the serum of LDL cholesterol up to 2%, respectively [4]. According to Kris-Etherton, isocaloric replacement of 5% energy obtained from saturated fatty acids with polyunsaturated fatty acids (PUFA) (such as oleic acid) will reduce coronary heart disease up to 40% [5]. This goal can be achieved by altering dietary patterns and modifying the foods naturally rich in saturated fatty acids with polyunsaturated fatty (such as oleic) acids [6]. Because Omega-3 of LCPUFAs (long-chain polyunsaturated fatty acids) lower cholesterol and prevent coronary heart diseases, they provide widespread nutritional and health benefits [7-9]. The most important omega-3 LC PUFAs including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) mainly originate from fish oil. While these acids reduce lipoproteins, blood pressure, cardiac function, endothelial function, vascular reactivity and cardiac electrophysiology, they have anti-platelet and anti-inflammatory effects in human body [10]. Various studies have recommended that the daily dose of EPA and DHA for an average person is approximately 300 mg (190-330 mg) and represents the consumption of two portions of fish meat (one oily) per week. Various Health Agencies AHA, AFSSA, Health Council of

the Netherlands recommended using 2-10 g/day of oleic acid (derived from mainly olive oil) instead of using milk fat [2,11,12]. It represents only 1-4% of the energy in a 2500 kcal diet. Milk proteins (used as surfactants in many formulated food) are adsorbed rapidly either as individual molecules or in the form of aggregates at the new oil/water interface during emulsification. Overall, it is a good nutritional strategy to substitute the milk fat of dairy products with a combination of olive and fish oils and reduce the intake of saturated fatty acids in favor of healthier fatty acids [6,13]. Whey proteins (β -lactoglobulin, α -lactalbumin, bovine serum albumin and immunoglobulin) are characterized by well-defined three-dimensional structures held together by disulphide bridges, and they are much more rigid than caseins [14]. Since whey protein and casein can efficiently stabilize fish oil suspension and have anti-oxidative function in an emulsion system they can be replaced with milk fat successfully in low fat dairy products [15]. When Martini et al. [3] fortified a 50% reduced fat cheddar cheese with low levels of n-3 fatty acids (18 and 35 mg of DHA and EPA per serving size), its samples did not develop a noticeable fishy flavor compared with the control, whereas at the highest fortification level (71 mg of DHA/EPA per serving size) the fishy flavor was recognized easily. Cumin seed powder has been used for many years in various food products to generate pleasant flavor and also mask nutty (such as fishy)

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smells [16]. Ye et al. [15] reported that emulsion of fish oil with milk protein is a helpful complex carrier for elevating the fortification level of omega-3 LC PUFA in processed cheese products. Aryana substituted 25, 50, and 100% of the milk fat with Omega Pure (a commercial oil rich in n-3 fatty acids) in cheddar cheese and found a significant improvement difference between the flavors of fortified cheese compared with the control samples during 2 months cold storage [17].

The objective of this study was to make a low fat fortified feta cheese with cumin powder, fish and olive oils and compare its fatty acid composition, micro-structure and rheological properties with the control sample after 0, 30 and 60 days of cold storage (or post-ripening time).

Materials and Methods

Materials

Fresh skim retentate was supplied by Pegah-e Khorasan Dairy Co. (Mashhad, Iran). Whey protein concentrate (WPC) was purchased from Milei Co. (Germany). Fish oil containing 15% EPA, 9% DHA and 30% total n-3 PUFA was supplied by DSM Co. (Switzerland). Commercial olive oil (containing 64.0 oleic, 14.5 linoleic, 17.0 palmitic, 3.5 stearic and 1.0 % α -linoleic acid) and cumin powder were purchased from a local market. Mesophil starter cultures (R-704) with a combination of *Lactococcus lactis* (subspecies of *cremoris*) and *Lactococcus lactis* (subspecies of *lactis*) were purchased from Christian Hansen Co. (Denmark). Rennet (Fromase™ TL) as a microbial coagulant (a product of *Rhizomucormiehei*) was purchased from DSM Food Specialties (Netherlands). All the chemicals were prepared from Merck Co. (Germany).

Cheese Making

According to Robinson and Bylund methods and processing systems of Tetra-Pak AB (Lund, Sweden), samples of ultra-filtrated (UF) feta cheese were made at Pegah-e-Khorasan Dairy Factory [18,19]. While the normal (or standard) and low fat UF-Feta cheeses have about 18% MSNF (milk solid non-fat), their fat contents are respectively around 16 and 8%. The retentate of skim milk (obtained by ultra-filtration), WPC, cumin powder, milk fat, fish and olive oils were mixed for few min with a Bosch mixer (Model MFQ-1620, Germany) in such a way that the final fortified feta cheese had 0.7% WPC, 0.5% cumin (beside 18% MSNF), 8.4% fat (5% milk fat+3% olive oil+0.4% fish oil which contains 10% of daily requirements of DHA and EPA). The control samples of UF feta cheese with 8.4% milk fat was made with the same procedure and without any other ingredient. Then the resulting mixture thoroughly blended, preheated in a water bath (Model WNB 10, Memmert GmbH+Co. KG, Germany) to 45°C, homogenized with an Ultra-turrax T25 (IKA, Germany) for 1.5 min at 20000 rpm, pasteurized in the same water bath at 74°C for 40s, and then cooled down to 32°C. After adding the required starter and rennet, the mixed samples were transferred into 100 g polypropylene cheese cups and incubated at 30°C for 20 min. When coagulation was done, fine salt powder (1.5% w/w) was spread on the top of thin parchment paper placed on each cheese cup and sealed with aluminum foil. When the pH of prepared cheese samples decreased to 4.8 at $30 \pm 1^\circ\text{C}$, they were transferred to a cold room at $5 \pm 1^\circ\text{C}$ for 3 days to complete the ripening time. Next, they were stored at the same conditions and the chemical analysis, textural measurement and sensory evaluation were performed on 0, 30, and 60 days of storage or post ripening time.

Physicochemical analysis

The important cheese quality indicators including pH, MSNF, fat,

protein and salt content of each sample was measured after ripening [20]. A digital pH-meter (Knick, 766 Calimatic, NielsBohrweg, Utrecht, Netherlands) and a vacuum oven were used to measure respectively the pH and total solids of cheese samples. The Kjeldahl (No. 920.123), AOAC and Mortensen et al. methods were used to determine respectively the protein, fat and fat's peroxide value in different cheese samples [21,22,23]. Exactly 10 g of each sample was transferred to a 500 mL plastic centrifuge tube and 200 mL of mixed chloroform-methanol (7:3) was added to separate the fat content. After homogenization (with Ultra-turrax T25), 50 mL of 1 mM CaCl_2 was added to the suspension and shaken for 10 s. Then the mixture was centrifuged at 1400 g and 20°C for 30 min and its supernatant was transferred to a 500 mL separation funnel to remove and collect its lower chloroform layer. The upper layer with its solid content was blended and washed two more times with chloroform (each time 150 mL). The three collected chloroform layers of each sample were mixed and transferred to a Buchi vacuum rotavapor R-215 (Buchi Labortechnik AG, Switzerland) to evaporate and remove the chloroform solvent from the dissolved fat at negative pressure of 0.1 MPa at 60°C. Then the remaining oil was weighed and flushed with nitrogen (to freeze) for further analysis. The International Dairy Federation [24] (IDF) Standard Method of 74A (1991) with some modification [25] (described by Drusch et al. 2007) was applied to determine peroxide value (PV) of lipids. After adding the iron (II) chloride and ammonium thiocyanate solutions to the fat samples, they were kept under dimmed light for 5 min and their absorbencies were read at 500 nm. The reading results were expressed as milli-equivalents of oxygen per kilogram of lipid in cheese samples. According to the American Society of Oil Chemists (AOCS) [26] Standard Method of Cd 19-90 (2009), 50 mg of each cheese sample was weighed in a 25 mL volumetric flask followed by dissolving with 1-butanol and adjusting its volume (up to 25 mL) to measure thiobarbituric acid reactive substances (TBRS). Later, a test tube was used to mix 5 mL of the thiobarbituric acid and 5 mL of the test solution for each cheese sample. After heating the test tube for 120 min in a thermostatic bath, it was cooled under running tap water for about 10 min until its temperature reached to 20°C. Then a 10 mm cuvette was used to read its absorbance at 530 nm.

Rheological properties

Static Measurement (hardness of texture): A universal testing machine (Testometric, M350-10 CT, England) was used with some modification [27,28] to measure hardness by uniaxial compression of cheese samples. A flat plunger with a 13 mm diameter was attached to the crosshead of this machine. The 100 g cheese cups were allowed to sit at room temperature for 4 h. Then each cheese cup was compressed uniaxially at a crosshead with depth speed of 30-80 mm min^{-1} and its hardness was calculated.

Dynamic measurements (storage modulus, loss modulus, and loss tangent): The method of Madadlou et al. [28] was employed to measure frequency sweep test by using a Universal Dynamic Spectrometer (Anton Paar, Physica MCR 301, Austria) with small amplitude oscillatory shear measurements. In this system, the primary viscoelastic terms including the storage modulus (G'), loss modulus (G''), and loss tangent (δ) were determined [29]. This device had two horizontal and parallel plates with diameter and gap size (trail thickness) of respectively 25 and 1 mm. The required samples were cut from the center of cheese blocks at 5°C, placed immediately in plastic bags, sealed and their temperatures were equilibrated to 201°C by keeping them in the Lab for at least 4 h. Then a small piece of each cheese sample was placed on the lower plate and the upper plate was slowly moved down until the gap size of 1 mm was obtained. The

extra cheese parts were trimmed off carefully with a razor blade and the sample kept for 15 min in the rheometer to relax and remove its stresses induced during sample handling. A strain sweep test at 0.1 Hz frequency was performed to obtain a linear viscoelastic range as the percentage of strain values varied from 0.01-2%. The linear region of strain (around 0.02%) was then selected and a frequency sweep test was performed from 0.1 to 10 Hz.

Microstructure

Based on the Drake et al. [30] method and its modifications suggested by Madadlou et al., cheese samples were prepared for scanning electron microscopy (SEM) at the 0, 30 and 60th day of storage time. The original sample of prepared fortified cheese was cut into 5-6 mm³ cheese cubes (by using a sharp razor), and immersed in aqueous solution containing 2.5% (w/w) glutaraldehyde and 1.25% paraformaldehyde for 3 h to fix the cheese cubes for scanning process. After two times washing (each time 10 min) the cheese cubes with 0.1M buffer of piperazine-N,N-bis, 2-ethanesulfonic acid (PIPES), they were dehydrated in a graded series of ethanol (40%, 55%, 70%, 85%, 90% and 96%) each one for 30 min followed by three times defatting in chloroform (10 min each). Later, the samples were pre-cooled in refrigerator at 4°C and submerged in liquid nitrogen to fracture them into approximately 1 mm pieces due to quick freezing [31]. The resulted pieces were then vacuum-dried to a critical moisture level and covered with gold for 6 min by using a sputter-coater (Balzers, Type SCD 005, BalTec Inc., Switzerland). The prepared samples were examined with a scanning electron microscope (XL Series, model XL30, Philips, Eindhoven, The Netherlands) operated at 20 kV and the structure images of cheese samples were recorded at 0.5K, 1K, 2.5K and 5K magnification levels.

Sensory evaluation

Based on the sensory abilities of panelists for detecting “fishy” odor in different food products, 10 trained panelists from the processing and R&D (research and development) sections of mentioned dairy plant were selected. About 15 g of each cheese sample was placed into a 50 g clear cup with a lid and conditioned at room temperature for 2 h before evaluation. Later, the panelists (seated in sensory booths with standard lighting) evaluated the appearance, aroma, flavor and texture of each cheese sample five times on a 5-point hedonic scale (1=least liked; 5=most liked).

Fatty acid composition

The method of Curtis was used to convert the fatty acids of cheese samples into methyl ester derivatives [32]. Then a GC/MS of Agilent Technologies 7890 (SGE Analytical Science, Germany) equipped with an ID- BPX 70 column (120m×0.25mm i.d) and a flame-ionization

detector was used to analyze these derivatives and identify the fatty acids profiles in each sample. The injector was operated in split mode (100:1 split ratio) and nitrogen was used as the carrier gas at a fixed linear velocity of 41.1 cm/s. The injector temperature was maintained at 250°C, and the total running time of each sample was 38 min. In this method the C19:0 synthetic triglyceride trionadecanoin was used as a surrogate spike to calculate analyte recovery, and the C23:0 methyl ester tricosanoic acid was used as an internal standard.

Statistical analysis

The cheese making experiment was carried out in triplicate using a complete randomized design. The general linear model (GLM) procedure of Minitab 16 software (English version, Minitab Inc., USA) was used to perform analysis of variance (ANOVA) and determine the differences among the means of resulting data at 5% significant level.

Results and Discussion

Physicochemical and sensorial characteristics

Table 1 show the effects of storage time on pH, MSNF, Fat, Protein, texture hardness and taste panel scores of fortified and control (unfortified) samples of low fat UF-feta cheese. The pH level of the fortified samples changed significantly within the 60 days of ripening. The curd acidification (an essential element) of cheese making was achieved by in-situ conversion of lactose to lactic acid because of its starter activity. The residual lactose was fermented relatively quickly to some extent depending on the salt/moisture (S/M) ratio of the curd. The increased osmotic pressure of aqueous phase (as a result of increasing S/M ratio) causes further dehydration and inactivation of bacterial cells (both desirable and undesirable) in the cheese, and inhibiting their growth. Although the amino acids released by proteolysis reaction cause a slight increase in pH during cheese ripening [33], overall the rate of lactic acid production and pH in the cheese making respectively increases and decreases [34]. However, the minerals bound to casein micelles increase buffering capacity of UF retentate and change the acidification kinetics of lactic acid bacteria. At low pH (<5.2), colloidal calcium phosphate of the cheese detaches from casein micelles and progressive breakdown of sub-micelles take place and convert them into small casein aggregates [35]. Subsequently, the bound water of small casein aggregates is released and helps them to dissociate. In the control sample the MSNF was decreased due to the rearrangement of casein network, formation of new cross-links bonds among the peptides bonds, disruption of fat globules and remaining of water in the network structure of feta cheese. However, in fortified samples the water was not trapped in the feta cheese structure because of using olive and fish oils and MSNF increased until the 60th day. Although the protein and MSNF

Physicochemical characteristics	Storage time (day)					
	0		30		60	
	Control	Fortified	Control	Fortified	Control	Fortified
pH	4.78 ± 0.02 ^a	4.7 ± 0.01 ^a	4.62 ± 0.03 ^b	4.48 ± 0.03 ^c	4.65 ± 0.01 ^b	4.46 ± 0.01 ^c
MSNF% ^b	18.2 ± 0.25 ^a	16.2 ± 0.28 ^b	17.6 ± 0.23 ^c	17.9 ± 0.24 ^c	17.2 ± 0.02 ^a	19.2 ± 0.31 ^d
Fat%	8.5 ± 0.02 ^a	8.5 ± 0.05 ^{ab}	8.7 ± 0.03 ^b	9.3 ± 0.0b ^c	8.7 ± 0.06 ^b	8.8 ± 0.08 ^b
Protein%	13.1 ± 0.25 ^a	11.5 ± 0.23 ^b	13.5 ± 0.17 ^a	11.9 ± 0.15 ^b	13.0 ± 0.12 ^a	12.1 ± 0.15 ^b
Texture hardness (N)	1.9 ± 0.03 ^a	1.7 ± 0.07 ^a	2.5 ± 0.04 ^b	2.3 ± 0.02 ^b	2.1 ± 0.06 ^{ab}	1.8 ± 0.05 ^a
Overall Acceptability ^c	4.2 ± 0.02 ^a	3.8 ± 0.02 ^{ab}	4.1 ± 0.06 ^a	3.9 ± 0.02 ^b	3.8 ± 0.08 ^b	3.2 ± 0.03 ^c

^aMeans with different superscripts within the same row differ significantly (P<0.05)

^bMSNF, milk solids nonfat, and

^cAveraged sensory panel scores for each cheese sample

Table 1: Effect of 5°C storage time on physicochemical properties and sensorial scores of fortified and control (unfortified) samples of low fat UF-Feta cheese^a.

of fortified and control cheese samples had significant differences in some cases, their differences were less than 2% even after 2 months of storage. According to Ye et al. [15] the casein is able to stabilize fish oil emulsions in fortified processed cheese curds successfully.

Rheological properties

Texture hardness: The cheese hardness is defined as the maximum force exerted on its cube to achieve 70% stress compaction respect to its original size [36]. Table 1 shows the texture hardness data and their changes for the fortified and control samples of the cheese within 60 days of storage time., The hardness of cheese samples increased significantly during the first month and then decreased in the second month of storage period. The increase of cheese hardness was more for control in comparison with fortified samples, most probably due to its

higher saturated fatty acids. Because of breaking sub-micelles into small casein aggregates [34] and significant pH decrease the cheese hardness increases until the first month of storage. In the 2nd month of storage, further breakdown of casein (proteolysis) happens and big molecules including large oligopeptides and peptides convert into small peptides and amino acids (proteinase and peptidase activities of lactic acid bacteria) and cheese texture become softer in this period [37]. Recently, researchers found a good correlation between softening of feta cheese and its proteolysis activity [28,38].

Storage modulus, loss modulus and loss tangent: Figures 1 and 2 show the log changes of G' and G'' in fortified and control samples of low fat feta cheese versus sweep test frequency. The G' and G'' moduli of fortified samples increased after 60 days of storage and reached to the similar values for the 0 day storage (fresh samples). While in pure elastic materials the stress and strain occur simultaneously in just one phase, in complete viscous materials, there is a phase difference between stress and strain, where strain lags stress by a 90 degree phase angle. The behavior of viscoelastic materials is somewhere in between of purely viscous and purely elastic materials that shows some phase lags in stress and strain [39]. The storage and loss moduli in viscoelastic materials measure the stored energy, representing the elastic portion, and the energy dissipated as heat, representing the viscous portion [39]. Once the elastic (or gel) character of a cheese sample dominates over its viscous (or liquid) behavior, its storage modulus (G') will be higher than loss modulus (G''). When this relationship is in reverse form, the viscous character dominates the elastic portion of a cheese sample [40]. In other words, the ratio of (G'/G'') is a good index for the elasticity and liquidity behavior in cheese samples. The rheological results showed that G' was greater than G'' and the elastic character was dominant in the fortified and control samples of feta cheese. Table 2 compares the results of G' and G'' obtained for fortified and control samples of low fat feta cheese during 2 months storage. While the elastic/viscous (G'/G'') ratios for fortified and control samples of feta cheese were very close to each other and changed from 1.14 to 1.17, the similarity of elastic and viscous characters of fortified cheese with control cheese was about 85% throughout the storage (Table 2). According to Lopez and Dufour, the poor texture of UF feta cheeses will be cured (due to high water binding capacity of whey proteins) as the cold storage or post-ripening is continued [41]. This is why the elastic behavior dominates over the viscous behavior. Karoui and Dufour implied that in a soft cheese sample, regions with the lower pH had higher G' and G''

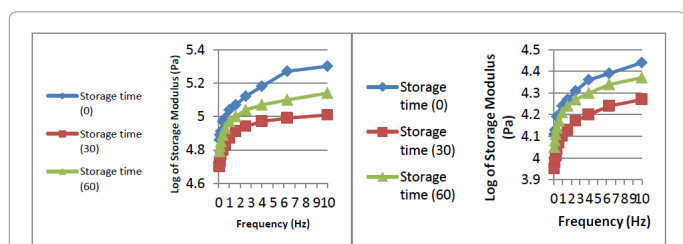


Figure 1: Effects of sweep test frequency on storage modulus (G') for the control (left side with Sd (mean values) = 0.015)* and fortified (right side with Sd (mean values) = 0.01)* samples of low fat feta cheese during storage.*These are the means of standard deviations for 0, 30 and 60 days of storage each one with 3 replicates.

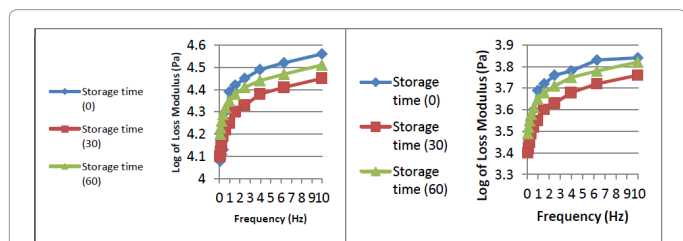


Figure 2: Effects of sweep test frequency on loss modulus (G'') for the control (left side with Sd (mean values) = 0.011)* and fortified (right side with Sd (mean values) = 0.010)* samples of low fat feta cheese during storage.*These are the means of standard deviations for 0, 30 and 60 days of storage each one with 3 replicates.

Muduli	Fort. Low Fat Feta (F)	Fort. Low Fat Feta (F)	Fort. Low Fat Feta (F)	Cont. Low Fat Feta (C)	Cont. Low Fat Feta (C)	Cont. Low Fat Feta (C)	(F/C)%	(F/C)%	(F/C)%
Days	0	30	60	0	30	60	0	30	60
G' (Min)	4.1	3.95	4.02	4.9	4.70	4.75	-	-	-
G' (Max)	4.44	4.27	4.38	5.3	5.00	5.15	-	-	-
G' (Ave)	4.27	4.11	4.20	5.08	4.85	4.95	-	-	-
$G'(Ave)_F / G'(Ave)_C^*$							84.1	84.7	84.9
G'' (Min)	3.45	3.40	3.48	4.25	4.15	4.50	-	-	-
G'' (Max)	3.85	3.76	3.82	4.55	4.45	4.18	-	-	-
G'' (Ave)	3.65	3.58	3.65	4.35	4.30	4.34	-	-	-
$G''(Ave)_F / G''(Ave)_C^{**}$							83.9	83.2	84.1
G'/G''	1.17	1.15	1.15	1.17	1.13	1.14	-	-	-

* and ** respectively are the elastic and viscous ratios of fortified feta cheese (F) to control (C) samples of low fat feta cheese

Table 2: The comparison of Storage Modulus (G') and Loss Modulus (G'') results for fortified and control samples of low fat feta cheese both with the total fat of 8.4%.

values and its softening texture was dependent to pH of product [42]. Wium (1997) reported that the lower pH of Feta cheese (4.5-4.7) was probably the main reason for its dominant elastic behavior [43]. The comparison of our results and similar data obtained from Karami et al. [1] (2009) for UF feta cheese with about 16% milk fat (and without any fish and olive oils) has been shown in Table 3. While the fat content of fortified cheese was almost half of full fat feta cheese (both kept for 2 months at cold store), their elastic to viscous ratios (G'/G'') were not completely dependent on the fat content and respectively were in ranges of (1.17-1.15) and (1.44-1.49). It can be concluded that the rheological similarities of fortified feta cheese in comparison with high fat feta cheese was about 80% (Table 3). Most probably the difference in rheological properties was related to the kind and content of the fats in two forms of feta cheese.

The rheological character of fortified cheese was evaluated by measuring the changes in the loss tangent (the index of melt-ability) of fortified cheese during cold storage. It was changed from 1.0 (dilute solutions) to 0.25 (amorphous polymers), and then 0.01 (glassy or crystalline gel) during 0, 30 and 60 days of cold storage (Figure 3). Since loss tangent (δ) was lower at lower frequencies, it indicates that the effective bonds (related to casein matrix) in feta cheese had more elastic characters at longer stress time. The cheese material is more liquid or solid when loss tangent (δ) is respectively bigger or smaller than 1.0. At

loss tangent (δ)=1, the cheese texture is in two equal portions of liquid and solid. Hence, changing the loss tangent (δ) to less than one signifies that the state of the material is crossing over from predominately liquid to solid [27]. Since our loss tangent data was around 0.2-0.3, the cheese texture of low fat feta cheese fortified with fish and olive oils was predominately in solid form regardless of storage time. In this case also the loss tangent of fortified cheese was about 80% of control samples of low fat feta cheese.

Micro-structure: Scanning electron micrographs of control and fortified samples stored at 0, 30 and 60 days showed in Figure 4. While the control samples had large protein aggregates with few pores, more pores with smaller protein aggregates were observed in fortified samples of feta cheese at zero day of storage (Figures 4a and b). After 30 days of storage, the numbers of cheese pores in control and fortified samples surrounded by the casein networks were increased considerably and less differences was noticed between them (Figures 4c and d). These holes were possibly the results of gas outlet (due to the fermentation) and water drainage that occurs during storage time. According to Fox the fermentation process (glycolysis, proteolysis and lipolysis) continues in the storage time and results more pores in the casein network [20]. Researchers hypothesized that these pores may be created because of the fat removal from feta cheese during storage [1,28,38,44]. According to Tunick, the low pH of feta cheese was the main cause for breakdown of the sub-micelles into non-linear strands of casein and finally to the aggregation of protein matrix in the cheese samples [29]. Different phenomena including disruption of fat globules and increasing the cross-links of among non-linear strands of casein changes the texture of feta cheese into an elastic form [45]. After 60 days of storage, many deep pores were formed in control and fortified samples of low fat feta cheese due to the proteolysis reactions and rearrangement of protein matrix and their pores structures became very alike to each other. In other words, the kind of fat (milk fat, olive oil or fish oil) did not make a noticeable change in the text structure of fortified cheese in comparison with control samples.

Oxidation of UF-Feta cheese fortified with fish oil : The peroxide value (PV) of both feta cheese samples (as an index of lipid oxidation) increased during the storage time (Figure 5). The shearing actions and turbulence of retentate during the processing made intense mechanical stresses, and agitation caused oxygen inclusion in the final

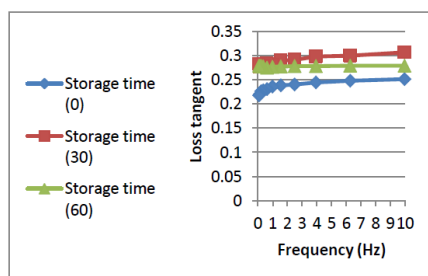


Figure 3: Effects of sweep test frequency on loss tangent (δ) of fortified samples of low fat feta cheese (with Sd (mean values) = 0.009*) during storage. *This is the mean of standard deviations for 0, 30 and 60 days of storage each one with 3 replicates.

Muduli	Fort. Low fat Feta (F)	Fort. Low fat Feta (F)	Fort. Low fat Feta (F)	High fat UF Feta (H)	High fat UF Feta (H)	High fat UF Feta (H)	F/H %	F/H %	F/H %
Days	0	30	60	0	30	60	0	30	60
G' (Min)	4.1	3.95	4.02	1.30	1.30	1.30	-	-	-
G' (Max)	4.44	4.27	4.38	1.60	1.77	1.84	-	-	-
G' (Ave)	4.27	4.11	4.20	1.45	1.54	1.57	-	-	-
G'' (Min)	3.45	3.40	3.48	0.84	0.84	0.84			
G'' (Max)	3.85	3.76	3.82	1.18	1.24	1.26			
G'' (Ave)	3.65	3.58	3.65	1.01	1.04	1.05			
G'(Ave)/G''(Ave)	1.17	1.15	1.15	1.44	1.48	1.49	-	-	-
[G'(Ave)_(F)/G''(Ave)_{(H)]x100}	-	-	-	-	-	-	81.3	77.7	77.2

Table 3: The comparison of Storage Modulus (G') and Loss Modulus (G'') results obtained from this study for fortified feta cheese with the total fat of 8.4% fat (5% milk fat + 3% olive oil + 0.4% fish oil) and Karami et al. (2009) data for the high fat UF feta cheese made with 16% milk fat and without adding any ingredient such as WPC, cumin powder, olive and fish oils.

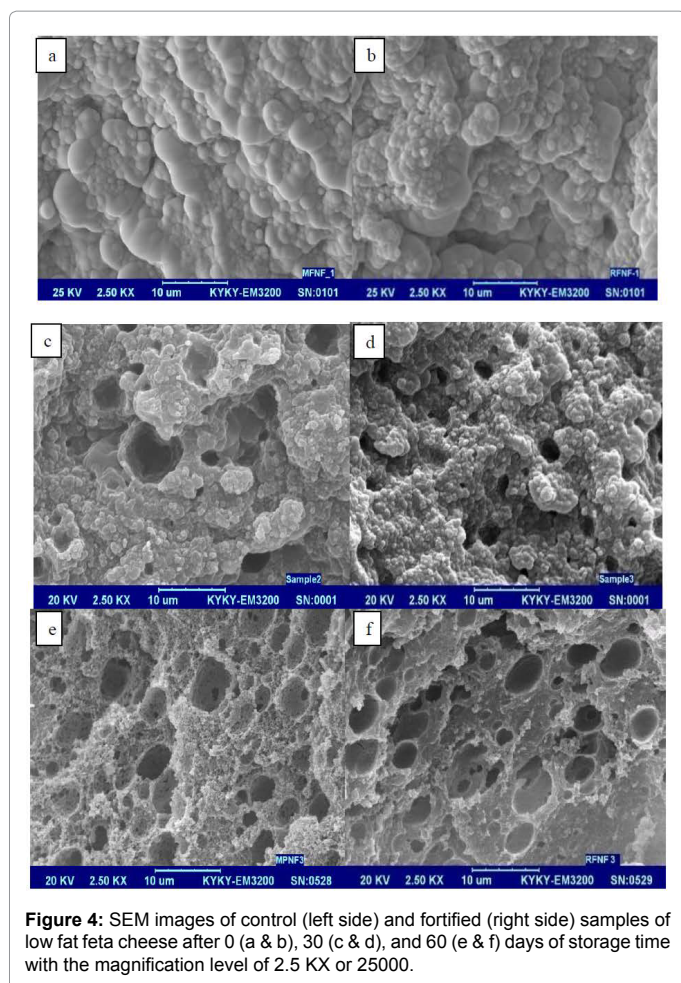


Figure 4: SEM images of control (left side) and fortified (right side) samples of low fat feta cheese after 0 (a & b), 30 (c & d), and 60 (e & f) days of storage time with the magnification level of 2.5 KX or 25000.

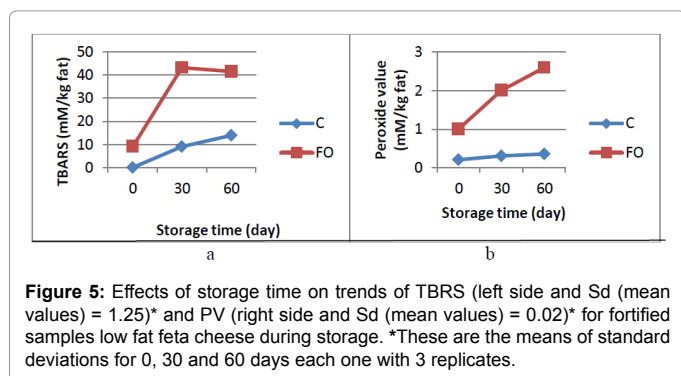


Figure 5: Effects of storage time on trends of TBARS (left side) and PV (right side) for fortified samples low fat feta cheese during storage. *These are the means of standard deviations for 0, 30 and 60 days each one with 3 replicates.

feta cheese product. In addition, disturbance of fish oil droplets (due to homogenization) helps the distribution of oxygen, catalysts and fat among the newly rearranged oil droplets, and also accelerates lipid oxidation [46,25]. On the other hand, the high level of polyunsaturated fatty acid (~35%) in cumin seed oil [15] was another reason for raising the PV. Lipid peroxides are cleaved thermally to yield secondary lipid oxidation products including low-molecular-weight aldehydes, ketones, alcohols, and short chain hydrocarbons [47]. The thiobarbituric acid reactive substances (TBARS) method was used to quantify the secondary lipid oxidation. The TBARS of samples increased noticeably after one month and then decreased slightly at the end of the 2 months storage time (Figure 5). Although the lipid oxidation in the fortified was higher

than control samples of feta cheese, and increased slightly because of omega-3 LC PUFA oxidation (as indicated by the changes in PVs and TBARS values), it was below 5 Milli Equivalent/Kg (the maximum standard levels specified by FDA for the food product consumption). Most probably this deficiency can be eliminated by using natural oil antioxidants. Ye et al. obtained similar results when they fortified processed cheese with fish oil [15].

Fatty acids profile: In this study the cheese samples were fortified with 30 mg or 10% of daily dose of EPA and DHA [2,11] (recommended by AHA 2006, and AFFSSA 2003). As Table 4 shows the total DHA and EPA content of fresh (zero day storage), fortified and low fat (8.4%) feta cheese had 1.2 % or (100 mg/100 g) or (1 mg omega-3/1 g). This Table shows that the reduction of omega-3 LC PUFA after 2 months cold storage time was only 12.5% which can be compensated in formula preparation. Although the fat content of both fortified and control samples were 8.4%, their fat comparisons showed that the 2 major saturated (Palmitic, and Myristic) fatty acids in fortified sample were respectively 35 and 52% less than the ones in control. On the other hand, the Oleic acid, EPA and DHA of fortified cheese were 147, 100 and 100% more than the control samples. Overall, the total unsaturated and saturated fatty acids of fortified cheese measured on 0, 30 and 60 days of storage were respectively 120, 124 and 156% more and 27, 28 and 29% less the similar control samples of feta cheese.

Sensory evaluation: The overall acceptability scores of control and fortified samples of feta cheese changed significantly during the 60 days of storage (Table 1). While the sensory scores of fortified sample was significantly lower than control samples at 0 day of storage, it improved considerably and reached to the similar scores of control samples (with no significant difference) after 30th day of cold storage. However, this score was changed significantly after the 60th day of storage mainly due to the appearance of surface yellowish color and unnatural flavor related to oxidation. Most probably the oil oxidation can be prevented by adding a natural antioxidant (such as β Carotene) to olive or fish oil before using them in cheese fortification. It has been revealed that the flavor of foods fortified with fish oil is the most sensitive indicator of their sensorial quality [48]. According to Ye et al. [15], the food with even very low fish flavor threshold significantly decreases its sensorial quality. However, they showed that the difference in the sensory perceptions of processed cheeses containing a low level of fish oil (5 g kg⁻¹) and the check sample containing no fish oil was not significant, perhaps because of masking property of fermented dairy products. Furthermore, no fishy off-flavor was detected based on the used level of fish oil (4 g kg⁻¹) and the sensory results showed that the fortified UF-Feta cheese had more than 70% acceptance and desirability. Kolanowski et al. [47] showed that in semi-solid dairy products like yogurt and cream, the fish oil level should be restricted between 1-5 g kg⁻¹. Whenever the fat content of the dairy product is increased the ratio of fish oil/ cheese fat can be increased accordingly [48]. Based on the results of taste-panelists, the texture and flavor of the cheeses were rated favorably after 30th day of storage most probably due to the addition of respectively WPC (as a protein provider) and cumin (high masking agent) powder. Overall, the taste panelists could not detect the fishy, oxidized or rancid flavor in fortified low fat UF-feta cheese samples mainly because of the masking efficiency of cumin seed oil. According to Ravi the cumin seed oil has a power to mask fish and rancid flavors due to its different volatile compounds including cuminal (8–17%), β -pinene (22–27%), β -myrcene (1.3–1.75%), p -cymene (23–39%), γ -terpinene (11–27%), p -mentha-1,4-dien-7-al (1.0–5.5%), and γ -Terpinene (26–28%) [49]. Martini and Ye used cumin seed powder in respectively cheddar and processed cheeses fortified with encapsulated

Fatty acid		Days of 5°C storage					
		0		30		60	
		Fortified	Control	Fortified	Fortified	Control	Control
C4:0	Butyric acid	3.15 ± 0.05	1.38 ± 0.01	2.95 ± 0.05	3.15 ± 0.05	1.16 ± 0.01	1.12 ± 0.01
C6:0	Caproic acid	1.35 ± 0.05	4.16 ± 0.02	1.23 ± 0.05	1.35 ± 0.05	4.05 ± 0.02	3.92 ± 0.02
C8:0	Caprylic acid	1.08 ± 0.02	2.87 ± 0.02	0.99 ± 0.02	1.08 ± 0.02	2.71 ± 0.02	2.69 ± 0.02
C10:0	Capric acid	1.45 ± 0.05	5.38 ± 0.05	1.29 ± 0.05	1.45 ± 0.05	5.36 ± 0.05	5.34 ± 0.05
C12:0	Lauric acid	3.1 ± 0.04	5.20 ± 0.04	2.99 ± 0.04	3.1 ± 0.04	5.13 ± 0.04	5.07 ± 0.04
C14:0	Myristic acid	7.2 ± 0.05	15.5 ± 0.05	7.12 ± 0.05	7.2 ± 0.05	15.31 ± 0.05	14.85 ± 0.05
C14:1	Myristoleic acid	1.09 ± 0.02	1.82 ± 0.02	1.07 ± 0.02	1.09 ± 0.02	1.75 ± 0.02	1.72 ± 0.02
C15:0	Pentadecanoic acid	0.91 ± 0.01	1.29 ± 0.01	0.85 ± 0.01	0.91 ± 0.01	1.23 ± 0.01	1.19 ± 0.01
C16:0	Palmitic acid	22.35 ± 0.05	34.43 ± 0.05	22.1 ± 0.05	22.35 ± 0.05	34.31 ± 0.05	33.99 ± 0.05
C16:1	Palmitoleic acid	2.29 ± 0.05	2.51 ± 0.05	2.2 ± 0.05	2.29 ± 0.05	2.36 ± 0.05	1.96 ± 0.05
C17:0	Margaric acid	0.57 ± 0.02	0.64 ± 0.02	0.47 ± 0.02	0.57 ± 0.02	0.49 ± 0.02	0.43 ± 0.02
C18:0	Stearic acid	9.21 ± 0.05	7.08 ± 0.05	8.18 ± 0.05	9.21 ± 0.05	6.8 ± 0.05	6.53 ± 0.05
C18:1t	Elaidic acid	1.72 ± 0.01	0.52 ± 0.01	1.62 ± 0.01	1.72 ± 0.01	0.61 ± 0.01	0.47 ± 0.01
C18:1c	Oleic acid	37.41 ± 0.05	15.12 ± 0.05	37.03 ± 0.05	37.41 ± 0.05	15.01 ± 0.05	14.94 ± 0.05
C18:2	Linoleic acid	4.97 ± 0.02	1.4 ± 0.02	4.75 ± 0.02	4.97 ± 0.02	1.39 ± 0.02	1.34 ± 0.02
C18:3	Linolenic acid	0.55 ± 0.01	0.65 ± 0.01	0.49 ± 0.01	0.55 ± 0.01	0.47 ± 0.01	0.34 ± 0.01
C20:1	Gadoleic acid	0.36 ± 0.05	ND	0.32 ± 0.05	0.36 ± 0.05	ND	ND
C20:5	Eicosapentaenoic acid (EPA)	0.75 ± 0.01	ND	0.72 ± 0.01	0.75 ± 0.01	ND	ND
C22:6	Docosahexaenoic acid (DHA)	0.45 ± 0.01	ND	0.39 ± 0.01	0.45 ± 0.01	ND	ND
	Total TFA (%)¹	1.72	0.52	1.62	1.72	0.61	0.47
	Total UFA (%)²	49.43	22.07	51.83	49.43	23.45	24.87
	UFA Addition³	121%	--	124%	121%	--	--
	Total SFA (%)⁴	50.37	77.93	48.17	50.37	76.55	75.13
	SFA Reduction⁵	27.6%	--	28.4%	27.6%	--	--
	PUFA (DHA+EPA)	1.2	--	1.11	1.2	--	--

¹Trans fatty acids, ² Unsaturated fatty acids (C14:1, C16:1, C18:1t, C18:1c, C18:2, C18:3, C20:1, C20:5 and C20:6), ³Saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0 and C18:0), ⁴ Poly unsaturated fatty acids (Docosahexaenoic acid and eicosapentaenoic acid), and ⁵ Not detectable.

Table 4: Fatty acid percentage in fortified and controlled samples of low fat (8.4%) feta cheese; (average of 3 replicates).

fish oil and obtained dairy products without noticeable fishy flavor [3,15].

Conclusion

This research showed that it is possible to fortify UF-Feta cheese with fish and olive oils to provide 10% of omega-3 LC PUFA as a daily requirement and with the whey protein concentrate (to increase hardness) and cumin powder (to mask the fishy and rancid flavor completely). Although the pH, MSNF, fat, protein, texture hardness and panel score of fortified feta cheese were significantly different from control samples in the first day of storage, its sensory scores and texture hardness became very similar (with no significant difference) to control samples after one month storage. The elastic/viscous (G'/G'') ratio of fortified feta cheese became very close (85%) to the same values in control sample. Although the PV of fortified cheese increased during the storage time it was below the maximum standard level of food products consumption (5 M equivalent/Kg). In addition, one month storage of fortified cheese did not make any negative effects on its physico-chemical, rheological, and sensorial properties. Furthermore, after two months of storage, the total saturated fatty acids and un-saturated (mainly oleic acid, EPA and DHA) fatty acids of the fortified samples were respectively about 30% less ($p < 0.05$) and 156% more ($p < 0.05$) than the control samples of low fat UF-feta cheese at similar storage time.

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Practical Application

The low fat ultra-filtrated feta cheese fortified with fish and olive oils had a good ability to be stored for two months at 5°C and did not show any negative effects on its physico-chemical, hardness (loss tangent (δ), storage modulus and loss modulus) and sensory criteria. While its total unsaturated (oleic acid, EPA and DHA) fatty acids was much more, and its saturated (palmitic, stearic and myristic) fatty acids were much less, its organoleptic criteria was very similar to common feta cheese. This research showed that it is possible to fortify feta cheese with 10% of omega-3 (long chain poly unsaturated fatty acids) as a daily requirement and the cumin powder had a good ability to mask completely its fishy flavor.

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