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The effects of commercial mixed-strain starter cultures on the chemical and sensory characteristics of UF-Feta cheese analogue during ripening

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Abstract

UF- Feta cheese is mostly produced from bovine milk and is usually consumed fresh or only after a short period of ripening (60 days). In this research, the influence of commercial starter cultures (SafeIT 2, FRC- 65 and R- 704) and ripening time (0- 60 days) on chemical (total solids, fat, protein, ash, salt, acidity, pH), biochemical (pH 4.6, TCA, PTA-soluble nitrogen, acid degree value) and sensory (color and appearance, aroma, texture, flavor and total acceptance) characteristics of UF- Feta cheese analogues was investigated. According to our results, the starter culture types were known to have a significant effect ($P\leq 0.05$) on pH, %salt, %protein, and pH 4.6- soluble nitrogen of cheeses, whereas the other chemical properties were not affected by them. Ripening time only significantly ($P\leq 0.05$) influenced %acidity, pH, %salt, acid degree value (meq acid 100 g^{-1} fat), %protein and %proteolysis products of samples. Also, the starter culture and ripening time did not affect the sensory properties significantly, excluding color and appearance, however, the produced cheeses from SafeIT 2 had higher sensory scores compared with the others containing FRC- 65 and R- 704 cultures.

Keywords: Analogue cheese, Lactic acid bacteria, Ripening time, Starter culture.

Introduction

The widespread use of lactic acid bacteria (LAB) in manufacturing fermented dairy products such as cheese, yogurt and cultured milk have been reported. Cheese, as one of the most important commercial fermented milk products, has received much scientific attention in recent years. The appearance, flavor, texture and overall acceptance of cheeses are considerably affected by LAB (Grattepanche *et al*,. 2007, Martínez-Cuesta *et al*,. 2001, Wouters *et al*,. 2002). LAB strongly influence cheese ripening process by lactic acid production, decrease in oxidation/reduction (OR) potential, autolysis and its associated release of intracellular enzymes, like protease and lipase, into the curd (Di Cagno *et al*,. 2003, Vernile *et al*,. 2008, Zárate *et al*,. 1997).

Cheese ripening is a complex process in which extensive microbiological, biochemical

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and physical changes occur continuously. Proteolysis and lipolysis are the two main biochemical reactions that occur during the cheese ripening period. Indigenous milk proteases and lipases, microbial enzymes and residual chymosin are responsible for these transformations. Peptides and free amino acids (FAAs) release during proteolysis directly and degradation of these compounds to related acids, amines, thiols, etc. indirectly affect the sensory properties of cheeses such as aroma, flavor and texture. During ripening, the lipolysis results in increased free fatty acid (FFA) concentrations, which in turn affects the textural and sensory characteristics of cheese samples. Hydrolysis of fat is especially important in soft cheeses (van Kranenburg *et al,*. 2002).

Many industrially utilized dairy starter cultures have a highly proteolytic activity (Korhonen and Pihlanto 2006). The sensory properties of cheeses are improved by LAB as a result of casein catabolism and produced peptides and FAAs (Beresford and Williams 2004). According to the literature, LAB applied as starter cultures have generally weak lipolytic activity (Larráyoz *et al,*. 2001, Sarantinopoulos *et al,*. 2001, Avila *et al,*. 2007), however, their high number or extended ripening time can release high amounts of FFA (Gobbetti *et al,*. 1997c, Santillo *et al,*. 2007, Sheehan *et al,*. 2009). LAB can also produce lactic acid even during ripening which is the principal cause of lowering the pH (Wang *et al,*. 2012).

Ultrafiltration (UF) technique has been widely used for the manufacture of soft cheese varieties, especially for UF-Feta cheese. This cheese, which is very popular in Iran, is mostly produced from bovine milk and is typically consumed fresh or only after a short period of ageing (60 days). UF-Feta cheese has a maximum shelf life of 2 months at refrigerated temperature (Iran Standard no. 12736).

In general, different dairy and non-dairy proteins, types of fats or edible oils, various types of starches and water are the most important ingredients in analogue cheeses formulations (Guinee, 2007). Inaccessibility to fresh milk or low milk production in some areas, lower price and variety of formulations are the most important reasons for extension of cheese analogues. Dairy (Milk protein concentrate (MPC), Whey protein concentrate (WPC), skim milk powder (SMP)) and non-dairy (soy, peanut) protein sources, dairy fat (cream, butter and butter oil) and vegetable oil or fat, salt and water are the main constituents which are used in the production of UF-Feta cheese analogue. The chemical compositions of this type of cheese are as follows: a minimum of 35% (w/w) total solids (TS), a minimum of 10% (w/w) protein, 10- 25%, 25- 45% and 45-60% (w/w, on dry basis) fat for low-, semi- and full fat types, respectively, a maximum of 3% salt, 0.5- 2% acidity (lactic acid) and a maximum of pH 5.2. (Iran Standard no. 12736). Besides the short ripening time, the use of vegetable fats and proteins in UF-Feta cheese analogues formulations is also described to enhance the texture and flavor problems in these cheeses. No data have previously been reported in the literature on the improvement of sensory properties of these cheeses during ripening. Therefore, the aim of this study was to determine whether the sensory characteristics of UF-Feta cheese analogue could be improved using starter cultures. On this basis, three commercial starter cultures (SafeIt 2, R-704 and FRC-65) were used and their effects on different properties of cheeses were evaluated during ripening.

Materials and methods

Calcium chloride (food-grade) was obtained from Kemira Agro Ltd. (Helsinki, Finland). MPC-75 (75% protein, 1.5% fat, 10.9% lactose, 7.6% ash, 5% water) was prepared from Milei GmbH (Stuttgart, Germany). WPC-35 (35% protein, 3% fat, 50.2% lactose, 7.2% ash, 4.6% water), SMP (skim milk powder) (36% protein, 1.35% fat, 50.8% lactose, 7.85% ash, 4% water) and butter (82% fat, 0.49% protein, 16% water) were purchased from a local dairy factory (Pegah, Mashhad, Iran). Margarine (80% fat, 0.5% protein, 18% water) was supplied by Behineh Wazin Co. (Mahgol^{TM}) (Tehran, Iran). Full fat soy flour (38% protein, 18% fat, 15% soluble carbohydrate, 15% non-soluble

carbohydrate, 13.7% water) was obtained from Soyan Toos Co. (Mashhad, Iran). Three freeze-dried mixed cultures of SafeIt 2 (*Lactococcus lactis* subsp. *cremoris, L. lactis* subsp. *lactis, Streptococcus thermophiles, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lb. helveticus*), FRC-65 (*Lb. delbrueckii* subsp. *bulgaricus, St. thermophiles, L. lactis* subsp. *lactic* and *L. lactis* subsp. *cremoris*) and R-704 (*L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*) (Chr. Hansens, Denmark) were used as starter. Fromase® 2200 TL, as fungal rennet, was prepared from DSM Co., The Netherlands.

Cheese manufacture

The formulation of cheese samples was based on the optimized formulation obtained from the previous study (Gholamhosseinpour *et al,*. 2014). In the optimal cheese formulation, the amounts of MPC, WPC, soy milk and margarine were 9.13%, 3%, 15% and 7.65%, respectively. The production of cheese samples was also performed according to the method as described in detail by Gholamhosseinpour et al. (2018). Chemical, biochemical and sensory analyses of cheese samples were carried out on days 3, 20, 40 and 60 after manufacturing.

Compositional analyses

Samples of cheese were analyzed in triplicates for total solids matter by oven-drying (AOAC 2005), fat content by the Gerber butyrometer technique (BSI 1989), ash by incineration at 550ºC (AOAC 935.42, 2005), salt concentration by the Mohr method (IDF 1988), acidity by titration method (AOAC 920.124, 2005) and protein content by the standard micro-Kjeldahl method (AOAC 920.123, 2005) using the Kjeltec Auto 1030 Analyzer. The pH of a 1:1 slurry of cheese in distilled water (Al-Otaibi and Wilbey 2006) was also measured with a Metrohm pH meter (Model 691, Herisau, Switzerland) after calibrating with fresh standard buffers at pHs 4 and 7.

Biochemical assays

Proteolysis

The concentrations of pH 4.6-soluble nitrogen (pH 4.6-SN), trichloroacetic acid-soluble nitrogen (TCA-SN) and phosphotungstic acid-soluble nitrogen (PTA-SN) were measured according to Messens et al. (1999), Reis et al. (2000) and Tavaria et al. (2003) with some modifications, respectively. The nitrogen contents of these fractions were determined using the standard micro-Kjeldahl technique (AOAC 920.123, 2005). The contents of pH 4.6-SN, TCA-SN and PTA-SN were expressed per unit mass of total nitrogen (TN).

Lipolysis

A slight modification of Nunez et al. (1986) method was used for evaluating the lipolysis degree of samples by assaying the Acid Degree Value (ADV). All Biochemical determinations were performed in triplicate.

Sensory evaluations

Sensory evaluation was done on cheese samples after 3, 20, 40 and 60 days of production by ten trained panelists. The 2× 2× 2 cm pieces of cheese were prepared and their codes were randomly determined. The panelists assessed cheese samples in a random order and washed their mouth before testing of each sample. Color and appearance, aroma, texture, flavor and total acceptance of cheese samples were evaluated based on a five-point hedonic scale (1=very poor; 5=excellent).

Statistical analyses

A complete randomized design was used to evaluate the effect of starter type and ripening time on the response variables. Analysis of variance (ANOVA) was carried out using the Minitab VERSION 16.2.3 statistical software package (MINITAB Inc., USA).

Results and discussion

Chemical Properties

Table 1 shows the average values of the main compositional criteria of cheeses for the experimental treatments. The average amount of TS in cheeses prepared with different starter cultures was varied from 35.88% to 35.92%. According to our results, the TS content of the resultant cheese samples was not significantly affected by the various starter cultures. This is

consistent with the results of Hynes et al. (2003) and Hayaloglu et al. (2005), who stated that total solids content of cheese samples was not significantly affected by the type of starter. Changes in the amount of total solids during the ripening period of cheeses was between 35.83 to 35.94%. It was also observed that the amount of TS of each cheese samples was not significantly different during ripening. Miočinović et al. (2011) also did not observe any significant effect of ripening time on TS content. It should also be noted that there are contradictory reports about the effect of ripening time on TS content in different cheese varieties. Aly (1995), Azarnia et al. (1997), Al-Otaibi and Wilbey (2004) and Lopez et al. (2007) reported that with increasing ripening time, the amount of TS increased. They stated that the high temperature of curd preservation, which affects the hydration of casein, led to increase in the degree of syneresis due to increasing salt concentration in the curd; water evaporation during storage and low water absorption in cheeses with low pH could be the causes of this increase. Inversely, Milci et al. (2005) and Shahab Lavasani et al. (2012) reported a decrease in TS content during ripening. The migration of water-soluble proteins and peptides from the curd into the surrounding brine, the lipolysis and the transfer of FFAs from the block of cheese to brine have been suggested as the most important reasons for this decrease.

The amount of fat in cheeses containing different starter cultures ranged from 15.96 to 16.08% and at different ripening times between 15.94 to 16.11%. Based on our results, there was no significant impact of type of starter, ripening time and their interactions on the concentration of fat in cheese samples. Hynes et al. (2003) also reported no significant effect of starter type on the amount of fat in different cheeses. Various results have also been reported about changes in the concentrations of fat in different cheeses during ripening. Milci et al. (2005) and Shahab Lavasani et al. (2012) observed that as the ripening time increased, the fat content of the cheese samples decreased significantly. Fat hydrolyzed to FFA and volatile compounds has been stated as the main reason for this reduction. In contrast, Aly (1995) observed that the fat content of Feta cheese increased during ripening and stated that the decrease in moisture with time is the reason. Furthermore, Karami et al. (2009b) observed no significant differences in the fat concentrations of cheeses.

While the titratable acidity of cheeses was not significantly influenced by various starters, this parameter was significantly increased ($p\leq0.05$) during ripening for all samples so that the acidity from 0.95% on day 3 increased to 1.1% on day 60 (at the end of ripening). This increase in acidity has been attributed to the lactate formation and also produce free fatty and amino acids by lipolysis and proteolysis, respectively (Özer *et al*,. 2003, Souza and Saad 2009). Lactic acid catabolism and its entry into brine as well as ammonia production can reduce the acidity at the end of the ripening period in some cheeses. The interaction between the two factors (starter type and time of ripening) was not also significant. Depending on starter type, Hayaloglu et al. (2005) and Hayaloglu et al. (2013) reported both significant and non-significant effects of the starters on acidity of different cheeses.

The pH of the cheeses prepared with R-704 and FRC-65 starter, didn't show any significant differences. However, the pH of both these cheeses was significantly ($p\leq0.05$) different from those prepared with SafeIt 2 starter. Hayaloglu et al. (2005) did not find significant impacts of starters on the pH of various cheeses. The pH of the cheeses also differed significantly ($p\leq0.05$) over the ripening period. The pH trend of cheeses decreased after 40 days of production due to the conversion of lactose to lactic acid by LAB. Later on, an increase was observed until the end of the 60th day of ripening which could be as a result of the production of ammonia (common end product of amino acid catabolism) combined with the metabolism of lactic acid by yeasts and molds (Gobbetti *et al*,. 1997a, Gobbetti *et al*,. 1997b, Hayaloglu *et al*,. 2007). The two factors had no significant interaction effect on the pH.

Table 1- The effects of starter culture type, ripening time and their interactions on chemical properties of UF-Feta cheese analogues

Chemical and biochemical properties	Starter type	Ripening time (day)				Mean
		3	20	40	60	
Total solids (%)	SafeIt 2	35.83± 0.29	36.00± 0.00	35.67± 0.29	36.00± 0.00	35.88± 0.16
	R-704	36.00± 0.00	35.83± 0.29	36.00± 0.00	35.83± 0.29	35.92± 0.10
	FRC-65	36.00± 0.00	35.83± 0.29	35.83± 0.29	36.00± 0.00	35.92± 0.10
	Mean	35.94± 0.10	35.89± 0.10	35.83± 0.17	35.94± 0.10	
Fat content (%)	SafeIt 2	16.00± 0.00	16.00± 0.00	16.17± 0.29	16.17± 0.29	16.08± 0.10
	R-704	16.00± 0.00	16.00± 0.00	15.83± 0.29	16.00± 0.00	15.96± 0.08
	FRC-65	16.00± 0.00	16.00± 0.00	15.83± 0.29	16.17± 0.29	16.00± 0.14
	Mean	16.00± 0.00	16.00± 0.00	15.94± 0.20	16.11± 0.10	
Titratable acidity (% lactic acid)	SafeIt 2	0.93± 0.02	1.00± 0.00	1.03± 0.02	1.09± 0.02	1.01± 0.07
	R-704	0.96± 0.00	1.00± 0.00	1.02± 0.02	1.11± 0.02	1.02± 0.06
	FRC-65	0.95± 0.02	1.00± 0.00	1.03± 0.02	1.09± 0.02	1.02± 0.06
	Mean	0.95^{C}± 0.01	1.00^{B}± 0.00	1.02^{B}± 0.00	1.10^{A}±0.01	
pH	SafeIt 2	4.70± 0.01	4.67± 0.01	4.63± 0.01	4.76± 0.01	4.69^{A}± 0.05
	R-704	4.68± 0.01	4.65± 0.00	4.63± 0.02	4.73± 0.01	4.67^{B}± 0.05
	FRC-65	4.68± 0.02	4.65± 0.01	4.63± 0.01	4.75± 0.03	4.68^{B}± 0.06
	Mean	4.69^{B}± 0.01	4.66^{C}± 0.01	4.63^{D}± 0.00	4.75^{A}± 0.01	
Ash (%)	SafeIt 2	2.78± 0.19	2.78± 0.19	2.67± 0.00	2.67± 0.00	2.73± 0.06
	R-704	4.78± 0.19	2.67± 0.00	2.67± 0.00	2.67± 0.00	2.70± 0.05
	FRC-65	2.78± 0.19	2.78± 0.00	2.67± 0.19	2.78± 0.19	2.75± 0.05
	Mean	2.78± 0.00	2.74± 0.06	2.67± 0.00	2.71± 0.06	
Salt (%)	SafeIt 2	1.64^{de}± 0.00	1.66^{cd}± 0.03	1.76^{a}± 0.00	1.76^{a}± 0.00	1.70^{A}± 0.06
	R-704	1.60^{ef}± 0.02	1.69^{bc}± 0.02	1.69^{bcd}± 0.02	1.76^{a}± 0.00	1.68^{B}± 0.07
	FRC-65	1.56^{f}± 0.02	1.65^{cd}± 0.02	1.72^{ab}± 0.02	1.76^{a}± 0.00	1.67^{B}± 0.09
	Mean	1.60^{D}± 0.04	1.67^{C}± 0.02	1.72^{B}± 0.03	1.76^{A}± 0.00	
Protein (%)	SafeIt 2	10.60^{ab}± 0.02	10.47^{def}± 0.03	10.42^{fg}± 0.03	10.52^{bcd}± 0.03	10.50^{A}± 0.07
	R-704	10.58^{abc}± 0.02	10.45^{def}± 0.03	10.40^{fg}± 0.02	10.51^{cde}± 0.02	10.49^{A}± 0.08
	FRC-65	10.61^{a}± 0.02	10.45^{def}± 0.04	10.35^{g}± 0.04	10.44^{ef}± 0.03	10.46^{B}± 0.11
	Mean	10.60^{A}± 0.01	10.46^{C}± 0.01	10.39^{D}± 0.04	10.49^{B}± 0.05	
pH 4.6-SN/TN (%)	SafeIt 2	5.46^{d}± 0.00	5.74^{d}± 0.13	6.37^{c}± 0.27	6.83^{ab}± 0.00	6.10^{A}± 0.62
	R-704	4.00^{e}± 0.11	5.83^{d}± 0.08	6.75^{abc}± 0.03	6.99^{ab}± 0.07	5.89^{B}± 1.36
	FRC-65	3.07^{f}± 0.00	6.62^{bc}± 0.27	7.02^{a}± 0.03	7.01^{ab}± 0.06	5.93^{B}± 1.92
	Mean	4.18^{D}± 1.20	6.06^{C}± 0.48	6.71^{B}± 0.33	6.94^{A}± 0.10	
TCA-SN/TN (%)	SafeIt 2	1.59± 0.00	1.71± 0.00	1.77± 0.14	1.81± 0.08	1.72± 0.10
	R-704	1.55± 0.04	1.67± 0.05	1.79± 0.04	1.84± 0.00	1.7± 0.13
	FRC-65	1.58± 0.02	1.67± 0.05	1.79± 0.04	1.88± 0.05	1.73± 0.13
	Mean	1.57^{C}± 0.02	1.68^{B}± 0.02	1.78^{A}± 0.01	1.84^{A}± 0.04	
PTA-SN/TN (%)	SafeIt 2	0.07± 0.00	0.13± 0.00	0.21± 0.03	0.27± 0.01	0.17± 0.09
	R-704	0.07± 0.00	0.13± 0.01	0.19± 0.02	0.26± 0.01	0.16± 0.08
	FRC-65	0.07± 0.00	0.14± 0.01	0.20± 0.01	0.28± 0.02	0.17± 0.09
	Mean	0.07^{D}± 0.00	0.13^{C}± 0.01	0.20^{B}± 0.01	0.27^{A}± 0.01	
ADV (meq acid 100 g^{-1} fat)	SafeIt 2	0.11± 0.01	0.13± 0.03	0.15± 0.01	0.18± 0.01	0.14± 0.03
	R-704	0.11± 0.01	0.14± 0.02	0.16± 0.03	0.18± 0.01	0.15± 0.03
	FRC-65	0.10± 0.01	0.14± 0.00	0.16± 0.01	0.19± 0.01	0.15± 0.04
	Mean	0.11^{C}± 0.00	0.14^{B}± 0.01	0.16^{B}± 0.01	0.18^{A}± 0.01	

Means within a same row or column with different superscript lowercase and capital letters indicate significant differences ($p \leq 0.05$).

The amount of ash in samples containing different starter cultures varied between 2.70 to 2.75% and at different ripening times ranged from 2.67 to 2.78%. The results showed that the effect of starter, time of ripening and the interaction between them on % ash content of cheeses was not significant. Al-Otaibi and Wilbey (2004) and Hayaloglu et al. (2005) also reported that the starter type and ripening time did not significantly affect the ash content of cheeses.

The results obtained from the study showed that the effect of starters, ripening time and their interaction on the salt level was significant ($p\leq0.05$). The salt content of samples was 1.6%, 1.67%, 1.72% and 1.76% on 3, 20, 40 and 60-day, respectively. Azarnia et al. (1997) stated that this increase may be due to the loss of water as well as the gradual salt penetration from brine into the cheese during ripening. According to some authors (Hynes *et al*,. 2003, Muir *et al*,. 1996), the starter effect on the salt concentrations of various cheeses was not significant, while Hayaloglu et al. (2013) reported the significant effect of starter type on the salt content of Gokceada cheese. Different results have been reported about changes in the concentration of salts during ripening. No significant increase was observed by Azarnia et al. (1997) and Shahab Lavasani et al. (2012) in the salt concentration of cheeses during ripening but Karimi et al. (2012) stated a non-significant decrease in the salt concentration. Also, in some cases, there was no noticeable change in salt content during ripening (Al-Otaibi and Wilbey 2004, Karami *et al*,. 2008).

Proteolysis

According to Table 1, the content of protein in the samples was significantly ($p\leq0.05$) affected by the type of starter, ripening time and the interaction between them. The amount of protein in the cheeses decreased gradually up to 40 days of ripening and afterwards increased continuously until the end of the ripening process. The initial decrease (up to 40 days of ripening) in protein content can be attributed to the proteolysis, whereas the subsequent increase may be due to the water evaporation and the increase in TS.

The pH 4.6-SN is used as a ripening extension index in cheese, which is a measure of proteolytic activity and reflects the amount of proteins and peptides that are soluble in water at pH value of 4.6, the isoelectric point of caseins (de Oliveira Carneiro *et al*,. 2020). The obtained results (Table 1) indicated that the concentration of pH 4.6-SN/TN was significantly ($p\leq0.05$) affected by the starter type, time of ripening and their interaction, however, no significant difference was found between the concentrations of pH 4.6-SN/TN in cheese samples prepared with R-704 and FRC-65 starters. The effect of starter culture on pH 4.6-SN/TN is related to the type of strain and its thermophilic and mesophilic nature. Increase in the pH 4.6-SN/TN ratio during ripening of UF-Feta cheese has been also observed in previous studies (Karami *et al*,. 2009b, Fathollahi *et al*,. 2010, Miočinović *et al*,. 2011). As the time of ripening increased, an increase in the amount of pH 4.6-SN/TN from 4.18% on day 3 to 6.94% on day 60, was observed. The comparison of 3-days cheeses was showed that the pH 4.6-SN/TN content of samples prepared with SafeIT 2 starter was higher than that of the others, which this could be due to the presence of *Lb. helveticus* in SafeIT 2 starter and its autolytic activity. Hannon et al. (2006) also stated that the lysis of *Lb. helveticus* in UF-cheese was started from the beginning of ripening but the onset and the extent of lysis were dependent on the strains and species used. Release of intracellular peptidases following autolysis of starter and nonstarter lactic acid bacteria (NSLAB) further contributes to proteolysis in cheese (Pillidge *et al*,. 2003).

The cheese ripening depth index (TCA-SN/TN) indicates the amount of low molecular weight nitrogenous substances accumulated during ripening period, that remains soluble in a 12% TCA solution (de Oliveira Carneiro *et al*,. 2020). According to the results, the level of TCA-SN/TN was significantly influenced by ripening time ($p\leq0.05$), whereas the starter type and its interaction with ripening time had not significant effects (Table 1). The study of

Hayaloglu et al. (2013) showed that a significant influence of starter type on TCA-SN/TN ratio can result from either species diversity of starter culture or from process conditions. No significant differences were found in TCA-SN/TN content between the cheese samples prepared with various starter cultures, although, its concentration in the cheeses made with SafeIt 2 and FRC-65 starters was higher than those of cheeses made with R-704 starter, which may be due to the presence of thermophilic bacteria (*Lb. helveticus*, *Lb. delbrueckii* subsp. *bulgaricus* and *St. thermophiles*) in two first starter cultures and their higher proteolytic activity. The overall proteolytic activity of lactobacilli has been found higher than that of *Lactococcus lactis* because lactobacilli possess additional peptidases and because their peptidases have higher expression levels. The presence of streptococcal peptidases indicated that these species also play a role in peptide degradation (Manso *et al*,. 2005). The TCA-SN/TN level of cheeses increased significantly ($p \leq 0.05$) with ripening time which is in accordance with previous studies (Al-Otaibi and Wilbey 2005, Azarnia *et al*,. 1997, Pezeshki *et al*,. 2011). The highest increase in the level of this fraction was observed during the first 40 days of ripening. Casein hydrolysis during ripening has been expressed as the main cause of the increase in the amount of TCA-SN/TN in different samples.

The free amino acid index, represented by the PTA-SN/TN ratio, reflects the further hydrolysis of small peptides into dipeptides, tripeptides, and free amino acids (Lacroix *et al*,. 2010). Similar to the trend described for TCA-SN/TN, the starter type and its interaction with ripening time had no significant impact on PTA-SN/TN ratio but the value of PTA-SN/TN increased significantly throughout ripening ($p \leq 0.05$) so that its level increased from 0.07% at 3 days to 0.27% at the end of ripening (Table 1). This increase is due to the hydrolysis of casein. The study of Hayaloglu et al. (2005) on Turkish white-brined cheese also showed that the type of starter had no significant effect on the amount of PTA-SN/TN. Also, other studies conducted on different types of cheeses (i.e., semi-hard cheese from high protein UF milk retentate, Feta, and low-fat UF) suggested that the ripening time led to increased level of PTA-SN/TN in cheese (Moatsou *et al*,. 2002, Miočinović *et al*,. 2011). The SafeIt 2 and FRC-65 starter cultures produced higher concentrations of PTA-SN than the R-704 starter in cheeses, which may be explained by the higher proteolytic activity or by the faster autolysis of thermophilic lactobacilli of two first starters. The earlier lysis of thermophilic bacteria accelerates the release of intracellular enzymes into the cheese matrix and thus increases proteolysis rate (Pappa *et al*,. 2006). Daly et al. (2010) reported that the rapid autolysis of *Lb. helveticus* during ripening and liberation of active peptidases in cheese causes increase in free amino acids and peptides concentration in whey.

Lipolysis

The presence of lipases in cheese may originated from the milk, rennet preparation, primary starter cultures, adjunct starter cultures, NSLAB and exogenous lipase preparations (Collins *et al*,. 2004). The results showed that the acid degree values (ADVs) significantly ($p \leq 0.05$) increased in all cheeses during ripening, while starter type and its interaction with time had not significant effect on ADVs (Table 1). Hayaloglu et al. (2005) observed that the use of various starter cultures caused different levels of lipolysis in Turkish white-brined cheese. As it is seen from Table 1, the ADV ranged from 0.11 at 3 d. to 0.18 at 60 d of ripening. The highest increase in ADV was also observed in the first 20 days of ripening. Georgala et al. (2005) and Atasoy and Türkoğlu (2009) also studied different cheeses and found that the level of ADV increased during ripening. Shahab Lavasani et al. (2012) Stated that the increase in ADV during ripening of Lighvan cheese was due to proteolysis and post-acidification. Also, the reason for the slowing down of lipolysis at the end of the ripening period of some cheeses is the inhibitory effect of increasing the concentration of salt and free fatty acids on the activity of

lipase (Azarnia *et al*,. 1997, Shahab Lavasani *et al*,. 2012).

Sensory properties

According to Table 2, only ripening time and its interaction with starter culture had significant effects ($p \leq 0.05$) on color and appearance scores of cheeses, whereas the type of starter culture did not show significant influence. The panelists awarded highest scores to 3 day old cheeses containing FRC-65 cultures and lowest scores to 60 day old cheeses made with starter R-704. The scores of color and appearance of cheeses prepared with SafeIt 2 and FRC-65 cultures decreased continually with storage time whereas these scores in cheeses made with starter R-704 increased during the first 40 days of ripening and then decreased thereafter. Hayaloglu et al. (2005) did not observe significant impacts of starters on the color and appearance of cheese. With regard to the ripening time, some reports found that this variable had no significant effects on appearance characteristics of cheeses (Milci *et al*,. 2005, Miočinović *et al*,. 2011).

Table 2- The effects of starter culture type, ripening time and their interactions on sensory properties of UF-Feta cheese analogues

Sensory properties	Starter type	Ripening time (day)				Mean
		3	20	40	60	
Colour & appearance	SafeIt 2	$4.50^{ab}\pm0.53$	$4.40^{ab}\pm0.52$	$4.20^{ab}\pm0.42$	$4.00^{ab}\pm0.67$	4.28±0.22
	R-704	$4.10^{ab}\pm0.32$	$4.40^{ab}\pm0.52$	$4.50^{ab}\pm0.53$	$3.90^{b}\pm0.57$	4.23±0.28
	FRC-65	$4.70^{a}\pm0.48$	$4.20^{ab}\pm0.42$	$4.10^{ab}\pm0.32$	$4.10^{ab}\pm0.32$	4.28±0.29
	Mean	$4.43^{A}\pm0.31$	$4.33^{A}\pm0.12$	$4.27^{AB}\pm0.21$	$4.00^{B}\pm0.10$	
Texture	SafeIt 2	4.50±0.71	4.40±0.52	4.30±0.67	4.20±0.42	4.35±0.13
	R-704	4.30±0.48	4.10±0.57	4.20±0.79	4.00±0.67	4.15±0.13
	FRC-65	4.30±0.48	4.30±0.48	3.60±0.70	4.00±0.47	4.05±0.33
	Mean	4.37±0.12	4.27±0.15	4.03±0.38	4.07±0.12	
Aroma	SafeIt 2	4.10±0.88	4.30±0.48	3.80±0.79	4.10±0.74	4.08±0.21
	R-704	4.00±1.15	3.80±0.63	4.00±0.67	4.10±0.57	3.98±0.13
	FRC-65	4.40±1.26	4.20±0.63	3.40±1.07	3.80±0.79	3.95±0.44
	Mean	4.17±0.21	4.10±0.26	3.73±0.31	4.00±0.17	
Flavor	SafeIt 2	4.10±0.74	4.00±0.47	4.00±1.05	4.00±0.67	4.03±0.05
	R-704	4.10±0.32	3.60±0.70	3.80±0.79	4.50±0.71	4.00±0.39
	FRC-65	4.20±0.63	4.00±0.82	3.70±0.95	3.80±0.63	3.93±0.22
	Mean	4.13±0.06	3.87±0.23	3.83±0.15	4.10±0.36	
Total acceptance	SafeIt 2	4.10±0.74	4.00±0.00	4.00±0.67	3.90±0.74	4.00±0.08
	R-704	3.30±1.06	4.00±0.67	3.60±0.84	4.00±0.67	3.73±0.34
	FRC-65	3.80±0.79	3.70±0.67	4.60±0.52	4.00±0.67	4.03±0.40
	Mean	3.73±0.40	3.90±0.17	4.07±0.50	3.97±0.06	

Means within a same row or column with different superscript lowercase letters indicate significant differences ($p \leq 0.05$).

Means within a same row or column with different superscript capital letters indicate significant differences ($p \leq 0.05$).

According to our results, the cheese samples prepared with SafeIt 2 starter cultures were more spreadable and less hard (softer), therefore, these cheeses received higher scores for texture compared to the cheeses made with two other starter cultures, although the differences were not significant ($p>0.05$) (Table 2). Katsiari et al. (2002) and Hayaloglu et al. (2005) also reported no significant changes in texture scores of cheeses made with different starter cultures. Ripening time, also, had no significant effect on texture scores. The decrease in moisture content during the ripening was the reason for the decrease in the

texture scores of the cheeses. Ghods Rohani et al. (2010), Karami et al. (2009a) and Miočinović et al. (2011) found no significant differences in texture scores during ripening, while Karimi et al. (2012) observed a significant increase in texture scores up to 30 days of ripening.

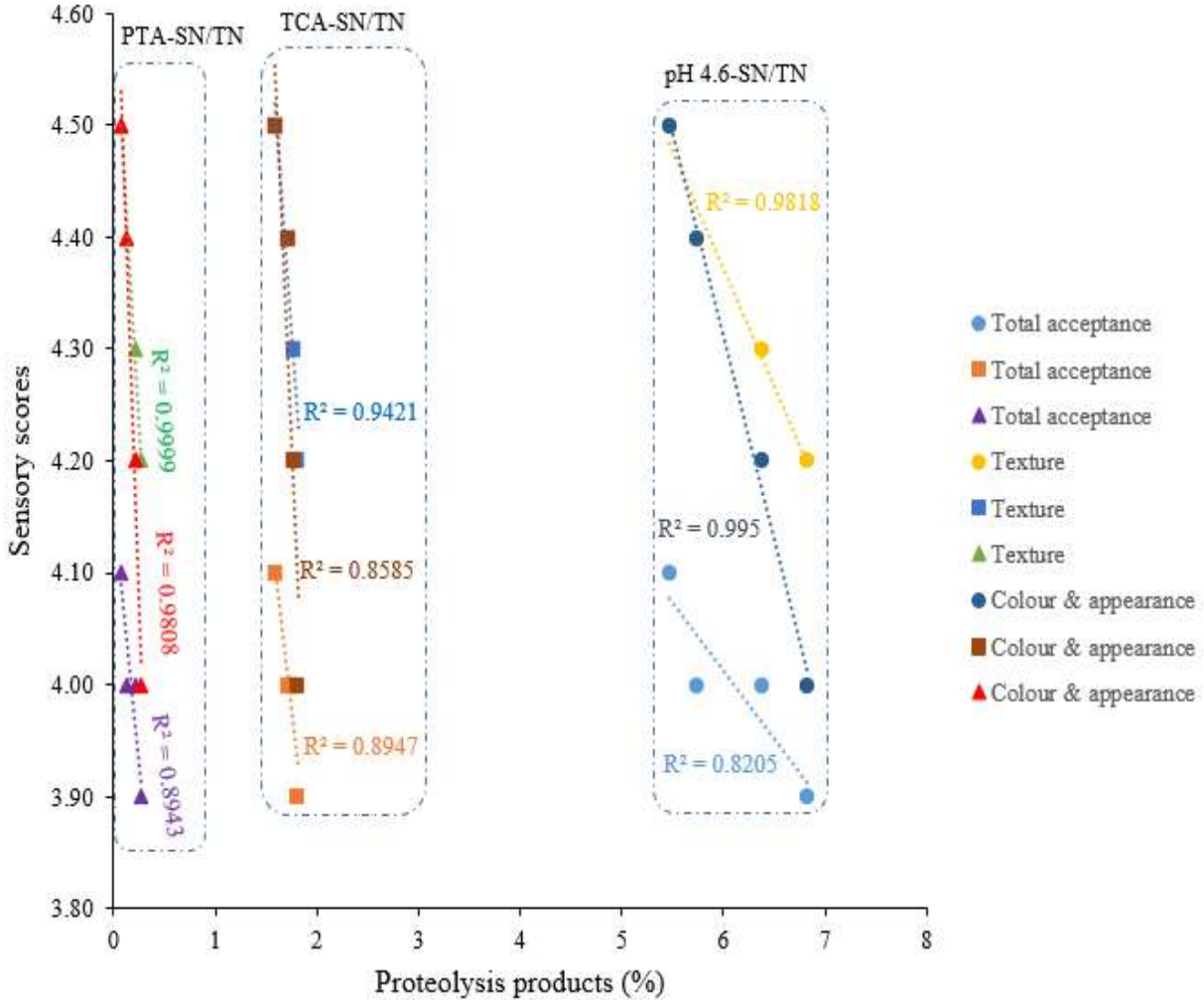

Fig. 1. Correlations between sensory scores (colour and appearance, texture and total acceptance) and proteolysis products (pH 4.6-SN/TN, TCA-SN/TN and PTA-SN/TN) of cheeses produced with SafeIt 2 starter culture.

Starter type, time of ripening and their interactions had no significant effect on aroma scores of cheeses (Table 2). The Hayaloglu et al. (2005) study also showed that the type of starter did not have a significant effect on the aroma scores of the cheese samples. Also, the results of the studies of Milci et al. (2005) and Miočinović et al. (2011) (up to 35 days of ripening) showed no significant differences for aroma scores of cheeses during ripening.

The flavor scores of cheeses were not significantly ($p>0.05$) influenced by starter type, ripening time and their interaction (Table 2). As it can be observed, the mean flavor scores of cheeses made with SafeIt 2 starters was higher than that of the cheeses prepared with R-704 and FRC-65 starter cultures. This could be due to the higher proteolytic activity of SafeIt 2 starters, leading to an increase in FAAs. Gomez et al. (1999) and Hayaloglu et al,. (2005) also did not report any significant impact of the starter type on the flavor scores of different cheeses. The effect of ripening time on the flavor scores of the cheeses was also not

found to be significant (Karami *et al,*. 2009a (from 20 d to 60 d of ripening), Ghods Rohani *et al,*. (2010) and Alonso *et al,*. (2011)). However, some studies showed a significant increase in flavor scores of different cheeses with ripening (Karimi *et al,*. (2012) (up to 60 d of ripening) and Shahab Lavasani *et al,*. (2012)).

As can be seen in Table 2, during the ripening period of 60 days, the total acceptance scores of cheeses produced from different starter cultures did not differ significantly ($p>0.05$). Similar to flavor scores, the mean total acceptance scores of cheeses produced with SafeIt 2 starters was also higher than that of the cheeses prepared with R-704 and FRC-65 starter cultures. In the study of Hayaloglu et al. (2005), no differences in total acceptance scores were also observed among the cheeses manufactured with different starter cultures. Karami et al. (2009a) found that the total acceptance scores of cheeses were not influenced by ripening time (from 20 d to 60 d), although these differences were significant in comparison to 3 d of ripening. In contrast to our findings, significant increase in total acceptance scores with time of ripening has been also reported ((Milci *et al,*. (2005); Karimi *et al,*. (2012) (up to 30 d of ripening) and Shahab Lavasani *et al,*. (2012)).

Good correlations were observed between sensory scores (color and appearance, texture and total acceptance) and proteolysis products (pH 4.6-SN/TN, TCA-SN/TN and PTA-SN/TN) of cheeses produced with SafeIt 2 starter culture (Figure 1), while no such correlations were found in the cheeses produced from the two other cultures (R-704 and FRC-65). Determination coefficients of mentioned attributes of cheeses containing SafeIt 2 starter were greater than 82%.

Conclusion

According to our results, pH, %salt, %protein and pH 4.6-SN were the only chemical characteristics that were affected by starter type and among them, pH 4.6-SN, as the main proteolysis product, was more important. However, the starter type had not significant effect on the sensory properties of cheeses, but the cheeses made with SafeIT 2, because of the presence of thermophilic and mesophilic strains and higher mean levels of proteolysis and lipolysis products, had higher sensory scores than those of cheeses produced from FRC-65 and R-704 cultures. Furthermore, high correlations (R^2= 0.8205- 0.9999) were found between some of the sensory properties and proteolysis products of cheeses produced from SafeIt 2 starter, while there were no such correlations for the cheeses containing R-704 and FRC-65. Therefore, for the manufacturing of UF-Feta cheese analogues, as the fresh unripened cheeses, the use of mixed commercial starter cultures contained combination of thermophilic and mesophilic strains are suggested instead of only mesophilic starter cultures. Ripening time, however, had no significant effects on %TS, %fat and %ash of cheeses, but the other chemical characteristics were influenced significantly ($p\leq0.05$). The sensory scores also were non-significantly reduced during ripening. From the results obtained in the present work, it may be concluded that the use of SafeIT 2 starter is preferred. It should be noted that the SafeIt 2 starter also has higher phage resistance stability than the two other starters.

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اثر کشت‌های آغازگر سویه‌مخلوط تجاری بر ویژگی‌های شیمیایی و حسی پنیر فتای فراپالایش آنالوگ طی دوره رسیدگی

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چکیده

پنیر فتای فراپالایش پنیری است که عمدتاً از شیر گاو تولید شده و معمولاً به‌صورت تازه و یا پس از یک دوره کوتاه رسیدگی (۶۰ روز) مصرف می‌گردد. در این پژوهش، اثر کشت‌های آغازگر تجاری (SafeIT 2، FRC - 65 و R- 704) و زمان رسیدگی (صفر تا ۶۰ روز) بر ویژگی‌های شیمیایی (مواد جامد کل، چربی، پروتئین، خاکستر، نمک، اسیدیته، pH)، بیوشیمیایی (ازت محلول در pH ۴/۶، اسید تری‌کلرواستیک و اسید فسفوتنگستیک، عدد اسیدی) و حسی (رنگ و ظاهر، آروما، بافت، طعم و پذیرش کلی) پنیر فتای فراپالایش آنالوگ بررسی گردید. بر اساس نتایج حاصله، نوع کشت آغازگر بر میزان pH، نمک، پروتئین و ازت محلول در pH= ۴/۶ نمونه‌های پنیر اثر معنی‌داری داشت ($P\leq 0.05$)، در حالی که سایر خواص شیمیایی تحت تاثیر معنی‌دار آن قرار نگرفت. اثر زمان رسیدگی نیز تنها بر میزان اسیدیته، pH، نمک، عدد اسیدی، پروتئین و محصولات پروتئولیز نمونه‌ها معنی‌دار بود. همچنین، کشت آغازگر و زمان رسیدگی بر خصوصیات حسی نمونه‌های پنیر، به‌جز رنگ و ظاهر، اثر معنی‌داری نداشتند، هرچند پنیرهای تولید شده از کشت آغازگر SafeIT 2 در مقایسه با نمونه‌های تولید شده از کشت‌های FRC- 65 و R- 704 امتیازات حسی بالاتری داشتند.

واژه‌های کلیدی: پنیر آنالوگ، باکتری‌های اسید لاکتیک، زمان رسیدگی، کشت آغازگر.

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