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Research Article
Improving the Quality of Ultrafiltered Ras Cheese using Mature Cheddar Cheese Slurry

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Abstract

Background and Objective: Ultrafiltered cheese was characterized by slow protein degradation, flavor development and hard texture. So, this study was aimed to improve the overall quality of UF-Ras cheese with adding mature Cheddar cheese slurry to ultrafiltered cow's milk (at a rate of 0, 1, 2 and 3% kg^{-1} retentates). **Materials and Methods:** Cheese quality was monitored during the 90 days of ripening by evaluating physicochemical, microbiological, microstructure and sensory attributes of control and experimental UF-Ras cheeses. One-way (ANOVA) and Principal Component Analysis (PCA) were used. **Results:** The ripening indices of 60 days UF-Ras cheese with cheese slurries were almost two-fold higher than of 30 days control cheese. PCA showed that the cheese samples were differentiated on the basis of ripening indices, for 90 days slurry-containing cheeses, for 60 days slurry-containing cheeses gathered with 90 days control cheese and for 30 days slurry-containing cheeses gathered with 30-60 days control cheeses. SEM micrographs of slurry-containing cheeses (60 days) and control cheese (90 days) were closely similar and no differences were seen between them. UF-Ras cheese made using mature Cheddar slurry received significantly ($p<0.05$) higher scores for flavor intensity, texture and overall quality compared to control cheese on the 1st month of ripening. **Conclusion:** These results suggest that the utilization of 2-3% of mature Cheddar cheese slurry to accelerate ripening and improving the overall quality of UF-Ras cheese.

Key words: UF-Ras cheese, accelerated cheese ripening, cheese slurry, principal component analysis, microstructure

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ras cheese, the most popular hard cheese made and consumed in Egypt, is conventionally manufactured from raw cow's milk without starter addition, that undergoes significant changes during ripening for at least 3-6 months to acquire desired sensory characteristics[1,2].

Ras cheese-making with ultrafiltration (UF) process has been documented in several reports[3,4]. One major drawback of this process in hard cheese-making, such as Ras cheese, is that the lack of typical texture, flavor and longer ripening period than the traditional ones. The longer ripening period in UF-Ras cheese was attributed to slow acidity development, relatively slower rate of proteolysis and lipolysis[4]. Several approaches have been used to accelerate cheese ripening and to improve its organoleptic characteristics[3,5]. Of these approaches suggested to increase ripening rate is adding cheese slurries to cheese milk or curd which were considered as a useful source of enzymes and microorganisms[6].

There is little published work on the acceleration of UF-Ras cheese ripening and to improve its textural and flavor development. Only one study had attempted to utilization of Ras cheese slurries to accelerate the ripening of UF-Ras cheese[7], while most studies have been carried out on using the starters, lipase and trace elements in the acceleration of UF-Ras cheese[8]. Cheddar cheese is the most popular cheese in worldwide and is rich in bioactive enzymes, lactobacilli and flavor precursors[9]. Cheddar cheese slurry can be effectively used for the acceleration proteolysis of UF cheeses with reduced ripening time[10].

Therefore, this work was carried out to accelerate UF-Ras cheese ripening using mature Cheddar cheese slurry as an attempt to improve the overall quality of UF-Ras cheese.

MATERIALS AND METHODS

Materials: Cow's milk was supplied by a private farm in Fayoum governorate, Egypt. Rennet powder (CHY-MAX, 2280 IMCU mL^{-1}, Ch. Hansen, Denmark), a yoghurt starter (DVS YC-X11) was obtained from Chr. Hansen's Laboratory (Copenhagen, Denmark). Calcium chloride and sodium chloride were obtained from a local market in Egypt. Mature Cheddar cheese for 12 months (5.32 pH, 37.50% moisture, 33.00% fat, 25.00% protein and 1.73% salt) was supplied by Egyptian importer. Experimental cheeses were carried out in winter 2016 at the Dairy Pilot Plant of Faculty of Agriculture, Fayoum University, Egypt.

MATERIALS AND METHODS

Mature Cheddar cheese slurry preparation: Cheese slurry was prepared by mixing grated mature Cheddar cheese (12 months old) with concentrated cow's milk in a ratio 2:1 at 45°C in an electric blender for 5 min. This homogenous slurry was used in UF-Ras cheese manufacture as a source of enzymes and microorganisms.

Experimental design and sampling: Whole cow's milk was concentrated 2-fold using Tech. Sep ultrafiltration unit equipped with Model tubular, carbon membrane (pasilac, silkburg, Denmark) at 50°C. For making UF-Ras cheese 2% of starter culture and 0.02% of $CaCl_2$ was added to the concentrated milk at 30°C. The 2x concentrated milk (80 kg) was divided into 4 equal portions. The 1st portion (20 kg) was left without additives (served as a control), while the other 3 portions were separately mixed with mature Cheddar cheese slurry at a rate of 1, 2 and 3% of UF retentate and served as T_1, T_2 and T_3, respectively. Coagulation by rennet addition (2 g/100 kg) and the procedure of cheese making was followed as described by El-Shibiny *et al.*[4].

Cheese ripening took place at 12±2°C and a relative humidity of 90-95% for 90 days. Cheeses were sampled in triplicate after salting stage at 15, 30, 60 and 90 days of the ripening period for some physicochemical, microbiological, microstructure and sensorial characteristics.

Physicochemical analysis: Grated cheese samples were analyzed for acidity, moisture, protein, fat and Water Soluble Nitrogen/ Total Nitrogen (WSN/TN) according to AOAC[11], total volatile fatty acids (0.1 N NaOH/100 g cheese) was determined according to Nollet and Toldra[12]. pH value was carried out on 10 g cheese sample dispersed in 10 mL of distilled water using a pH meter. The concentrations of free amino groups in cheese samples (mg leucine/g sample) were measured by Cd-ninhydrin analysis as described by Folkertsma and Fox[13]. All analyses were performed in triplicate and results reported as Means±Standard Deviation.

Microbiological analysis: Cheese samples were examined for the total bacterial counts using plate counts agar according to APHA[14], for the proteolytic and the lipolytic bacterial counts as described by Frank *et al.*[15]. Results were calculated as a log of mean colony-forming unit per gram of four cheese-making treatments.

Microstructure analysis: Cheese microstructure was studied by Scanning Electron Microscope (SEM) after 30, 60

and 90 days of ripening period according to McClements[16]. Samples were viewed with SEM (Model Quanta 250 FEG, Philips, Netherlands) attached with EDX-ray Unit, with accelerating voltage 30 KV.

Sensory evaluation: Cheese samples in cube shape (2×2×2 cm) were evaluated for flavor (60 points), body and texture (40 points) by 10 panelists of the staff members at Dairy Department, Faculty of Agriculture Fayoum University, according to the scoring sheet of El-Shibiny *et al.*[4].

All data were expressed as mean values±standard deviation and analyzed using one-way analysis of variance (ANOVA) followed by the Least Significant Differences (LSD) test at significant ($p<0.05$) using XLSTAT statistical software version 2007. The PCA was applied to the ripening indices data to investigate differences between the samples during the ripening period[17].

RESULTS AND DISCUSSION

Physicochemical characteristics: Some of the main physicochemical characteristics of control and experimental UF-Ras cheese samples during the ripening period are shown in Table 1. There were relatively higher moisture, fat and protein contents in slurry-containing cheeses than those of control cheese during the ripening period. This could be explained by the slurry added contained 33.00% fat and 25.00% protein, that significantly ($p< 0.05$) increased the fat and protein contents in UF-Ras cheese contained cheese slurry. Besides, higher moisture in slurry-containing cheese may be attributed to the high water-holding capacity of cheese slurry. This increase in the moisture increased the activity of lactic acid bacteria. Since all slurry-containing cheese exhibited significantly ($p<0.05$) higher acidity and lower pH values than in the control cheese and its acidity gradually increased as the level of cheese slurry increased. This could be attributed to the stimulation effect of soluble and nitrogenous compounds present in cheese slurry on the growth of lactic acid bacteria. Cheese acidity and pH values of the slurry-containing cheeses differed significantly ($p<0.05$) between cheeses of different slurry levels. The highest increasing values were found in UF-Ras cheese contained 3% slurry while the lowest values were recorded with UF-Ras cheese contained 1% slurry. These results are an agreement with these reported by Mostafa *et al.*[7].

Proteolysis and lipolysis progress evaluation: The variation in Water Soluble Nitrogen/Total Nitrogen (WSN/TN), free amino groups and Total Volatile Fatty Acids (TVFA) values of UF-Ras cheese treatments which were considered as indices of the ripening progress as shown in Table 2. There is a clear trend of increasing the WSN/TN, free amino groups and TVFA values gradually with the advance in ripening period in all treatments. As expected, adding cheese slurry to UF-cheese

Table 1: Physicochemical characteristics (Mean±Standard Deviation) of control and experimental UF-Ras cheeses during the ripening period

		Treatments			
			UF-Ras cheese with mature cheddar cheese slurry (%)		
Property	Ripening period (days)	Control	1.0	2.0	3.0
pH	15	5.70±0.01[a]	5.58±0.02[b]	5.57±0.02[b]	5.55±0.02[b]
	30	5.64±0.02[a]	5.59±0.01[b]	5.58±0.01[b]	5.57±0.01[b]
	60	5.60±0.01[a]	5.55±0.02[b]	5.45±0.02[c]	5.40±0.01[d]
	90	5.58±0.02[a]	5.40±0.02[b]	5.34±0.01[c]	5.30±0.02[d]
Acidity (%)	15	1.15±0.02[c]	1.33±0.01[b]	1.35±0.02[b]	1.38±0.01[a]
	30	1.28±0.02[c]	1.44±0.02[b]	1.48±0.01[b]	1.68±0.04[a]
	60	1.41±0.01[d]	1.73±0.02[c]	1.95±0.02[b]	2.10±0.01[a]
	90	1.57±0.02[c]	2.18±0.02[b]	2.31±0.05[a]	2.42±0.05[a]
Moisture (%)	15	37.08±0.03[d]	37.21±0.02[c]	37.43±0.02[b]	38.31±0.01[a]
	30	35.90±0.11[d]	36.12±0.02[c]	36.44±0.03[b]	37.29±0.02[a]
	60	35.35±0.01[d]	35.84±0.02[c]	35.90±0.05[b]	36.08±0.02[a]
	90	33.40±0.01[c]	34.49±0.01[b]	34.52±0.02[b]	34.73±0.03[a]
Fat (%)	15	34.10±0.10[d]	35.15±0.05[c]	36.30±0.05[b]	37.12±0.03[a]
	30	34.72±0.03[d]	35.65±0.05[c]	36.98±0.10[b]	37.80±0.10[a]
	60	35.55±0.05[d]	36.18±0.03[c]	37.52±0.03[b]	38.45±0.05[a]
	90	36.92±0.03[d]	37.18±0.03[c]	38.25±0.05[b]	39.19±0.01[a]
Protein (%)	15	24.27±0.04[c]	24.67±0.04[b]	24.86±0.04[a]	24.99±0.10[a]
	30	24.31±0.06[c]	24.86±0.04[b]	25.26±0.06[a]	25.29±0.04[a]
	60	24.48±0.04[c]	25.20±0.06[b]	25.52±0.06[a]	25.73±0.10[a]
	90	24.78±0.04[c]	25.48±0.07[b]	26.37±0.37[a]	26.56±0.10[a]

[a-d]Means of the same row with different superscripts differ significantly ($p<0.05$)

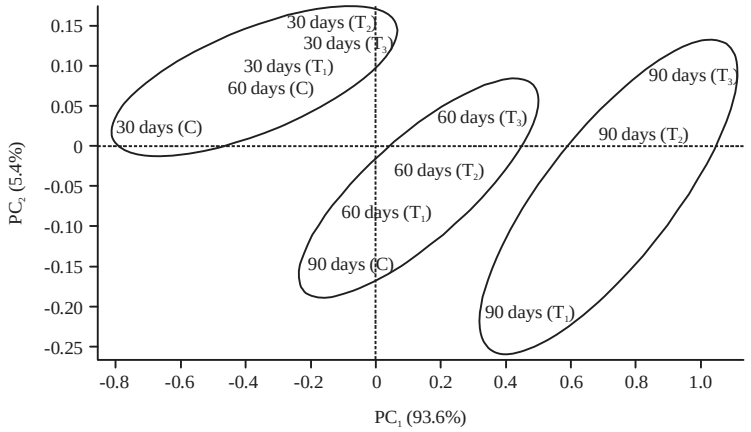

Fig. 1: PCA similarity map determined by PC_1 and PC_2 for ripening indices data of control and experimental UF-Ras cheeses during the ripening period

C: Control cheese, T_1: Cheese with 1% slurry, T_2: Cheese with 2% slurry, T_3: Cheese with 3% slurry

Table 2: Ripening indices (Mean±Standard Deviation) of control and experimental UF-Ras cheeses during the ripening period

		Treatments			
			UF-Ras cheese with mature cheddar cheese slurry (%)		
Ripening indices	Ripening period (days)	Control	1.0	2.0	3.0
WSN/TN (%)	15	7.01±0.15^{d}	7.59±0.10^{c}	8.21±0.20^{b}	9.02±0.18^{a}
	30	7.82±0.24^{d}	9.92±0.13^{c}	10.86±0.24^{b}	11.61±0.27^{a}
	60	8.43±0.14^{d}	11.14±0.24^{c}	12.33±0.12^{b}	12.81±0.19^{a}
	90	9.53±0.25^{d}	12.35±0.18^{c}	15.48±0.12^{b}	17.21±0.71^{a}
Free amino groups (%)	15	0.19±0.00^{d}	0.25±0.01^{c}	0.40±0.00^{b}	0.43±0.01^{a}
(mg leucine g^{-1})	30	0.26±0.00^{d}	0.41±0.01^{c}	0.44±0.00^{b}	0.58±0.01^{a}
	60	0.51±0.00^{d}	0.82±0.01^{c}	0.88±0.01^{b}	0.92±0.00^{a}
	90	0.80±0.01^{d}	1.21±0.01^{c}	1.24±0.01^{b}	1.35±0.02^{a}
Total volatile fatty acids (%)	15	16.21±0.04^{d}	22.70±0.01^{c}	26.40±0.02^{b}	30.50±0.01^{a}
(0.1 N NaoH/100 g)	30	22.41±0.02^{d}	30.46±0.04^{c}	33.48±0.03^{b}	38.45±0.05^{a}
	60	28.52±0.03^{d}	36.70±0.02^{c}	40.50±0.05^{b}	49.03±0.06^{a}
	90	33.22±0.03^{d}	43.20±0.02^{c}	60.50±0.01^{b}	76.04±0.07^{a}

$^{a-d}$Means of the same row with different superscripts differ significantly ($p<0.05$). WSN/TN: Water Soluble Nitrogen/Total Nitrogen, TVFA: Total volatile fatty acids

milk increased significantly ($p<0.05$) ripening indices values of UF-Ras cheese than that of control cheese and the increasing rates of those values was proportional to the amount added. These results could be attributed to the higher proteolytic and lipolytic activities in slurry-containing cheese. Furthermore, there was a significant difference ($p<0.05$) of WSN/TN, free amino groups and TVFA values between cheese of differing slurry levels. Apparently, the rate of ripening of UF-Ras cheese with slurry was higher than that of control UF-Ras cheese at the same ripening period. In general, the increasing rate of ripening indices was higher in cheese with 3% slurry followed by cheese with 2% and then 1% slurry which had lower nitrogen content than that of other treatments. This may be attributed to the high level of protease and peptidase activities with increasing the slurry levels. The increase in ripening indices values was almost two-fold higher in slurry-containing cheeses than of control cheese. Similar trends were obtained by other researchers Ammar *et al.*[18], Mostafa *et al.*[7] and Mehanna *et al.*[19].

Principal Component Analysis (PCA): In order to differentiate between cheese treatments according to their ripening indices values (WSN/TN, free amino groups and TVFA), PCA was applied to those values during the ripening period. PCA similarity map defined by the first two principal components of ripening indices values for UF-Ras cheese treatments during ripening as shown in Fig. 1. The total variance explained by the first two principal components was 99.00%. The WSN/TN, TVFA values were strongly correlated with the 1st Principal Component (PC_1), which explained 93.6% of the total variance, while free amino groups values contributed more strongly to the 2nd Principal Component (PC_2). Cheese

Table 3: Microbiological counts (mean log CFU g^{-1} ± standard deviation) of control and experimental UF-Ras cheeses during the ripening period

		Treatments			
			UF-Ras cheese with mature cheddar cheese slurry (%)		
Tests	Ripening period (days)	Control	1.0	2.0	3.0
Total bacterial counts	15	0.02±6.66^{c}	0.01±6.60^{d}	0.01±6.74^{b}	0.01±6.86^{a}
	30	0.02±6.35^{c}	0.01±6.31^{d}	0.00±6.56^{b}	0.00±6.76^{a}
	60	0.02±6.27^{c}	0.02±6.25^{c}	0.01±6.50^{b}	0.01±6.68^{a}
	90	0.02±6.02^{c}	0.01±5.90^{d}	0.02±6.08^{b}	0.01±6.32^{a}
Proteolytic bacterial counts	15	0.05±1.10^{c}	0.04±1.41^{b}	0.03±1.59^{ab}	0.03±1.75^{a}
	30	0.05±1.75^{d}	0.04±2.00^{c}	0.03±2.18^{b}	0.03±2.33^{a}
	60	0.03±1.88^{d}	0.04±2.04^{c}	0.04±2.19^{b}	0.02±2.41^{a}
	90	0.02±1.90^{d}	0.01±5.90^{c}	0.02±6.08^{b}	0.01±6.32^{a}
Lipolytic bacterial counts	15	0.10±1.20^{c}	0.03±1.59^{b}	0.03±1.75^{ab}	0.04±1.95^{a}
	30	0.10±1.42^{c}	0.03±1.88^{b}	0.03±1.94^{ab}	0.04±2.04^{a}
	60	0.06±1.67^{d}	0.03±1.97^{c}	0.01±2.31^{b}	0.02±2.45^{a}
	90	0.06±1.84^{d}	0.04±2.04^{c}	0.03±2.31^{b}	0.02±2.53^{a}

$^{a-d}$Means of the same row with different superscripts differ significantly ($p<0.05$)

samples are distributed into 3 main groups (30, 60 and 90 days) according to their ripening indices values. The slurry-containing cheeses (90 days) were clearly separated from the other cheeses and allocated in the right half of the PCA map according to PC_1. While slurry-containing cheese (60 days) and control cheese (90 days) were located in a separate group according to PC_1. The slurry-containing cheeses (30 days) and control cheese (30 and 60 days) were grouped at the upper left of the map. This was in agreement with the ripening indices of the cheese treatments which were made with slurry.

Microbiological quality: The total, proteolytic and lipolytic counts of UF-Ras cheese treatments during the ripening period are shown in Table 3. The total, proteolytic and lipolytic counts in slurry-containing cheese were higher significantly ($p<0.05$) than those in control cheese during the ripening period. This justifies our observation on higher acidity and ripening indices values of slurry-containing cheeses in comparison with control cheese (Table 1, 2). On the other hand, the total proteolytic and lipolytic counts increased gradually as the ripening period advanced in all treatments, while the total bacterial counts decreased. This trend was observed by other researchers[7,18].

Cheese microstructure: The SEM images of UF-Ras cheese treatments during the ripening period are shown in Fig. 2. The protein network in control cheese was more compact (dense clusters of casein particles) with very small pores compared with UF-Ras cheese with slurry, reflecting the hardness texture that was revealed by sensory evaluation. Whilst, SEM images for slurry-containing cheese showed many big pores maybe cause be the higher moisture and proteolysis rate in this cheese. The slurry-containing cheese (30 days) had an open structure that resembled the control cheeses (60 days). After 60 days of ripening, there were also similarities in the SEM images between the slurry-containing cheese (60 days) and the control cheese (30 days).

Sensory evaluation: The mean sensory scores of control and experimental UF-Ras cheese during the ripening period are shown in Table 4. The general trend of evaluation is as the ripening period advanced the sensory scores in all treatments increased gradually and this may be due to the progressive cheese proteolysis. It could also be noticed that the slurry-containing cheese gained the highest sensory scoring points than the control cheese during ripening period and the score was significantly ($p<0.05$) as increasing slurry levels.

The rate of improvement in cheese quality during ripening was slow in control cheese, while it was faster in slurry-containing cheese in particular with increasing the slurry level. This could be explained by increasing of protein degradation and fat hydrolysis in slurry-containing cheese which could be proved by increasing of all ripening indices values during the ripening period.

Less flavor scores in control cheese could be attributed to less soluble nitrogen, low free amino group sand volatile fatty acids. Similar trends were obtained by Ammar *et al.*[18]. Moreover, at 60 days the overall quality (flavor intensity, texture scores) of UF-Ras cheese made with 2 or 3% slurry was statistically superior to the control cheese at 90 days.

Overall, the slurry-containing cheese were preferred for all panelists and were characterized by smooth texture and flavor

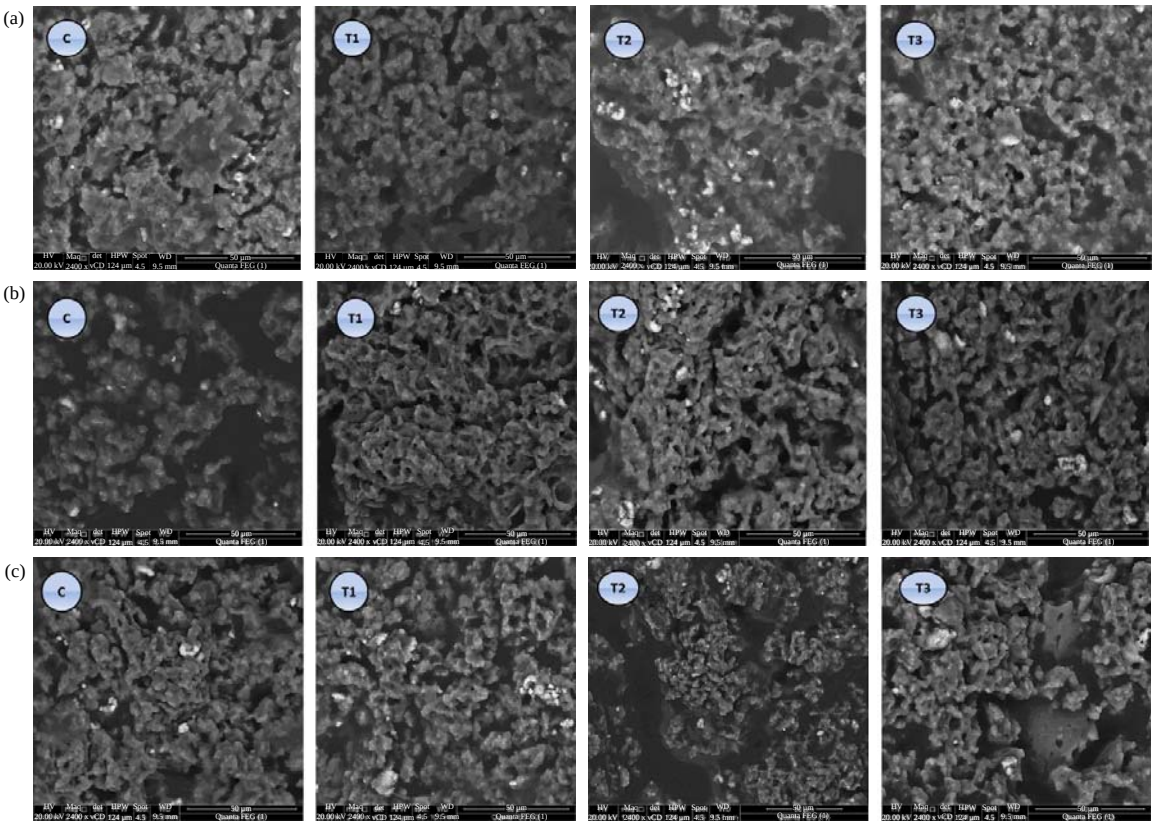

Fig. 2: SEM micrographs of control and experimental UF-Ras cheeses during the ripening period, (a) 30 days, (b) 60 days and (c) 90 days

C: control cheese, T_1: Cheese with 1% slurry, T_2: Cheese with 2% slurry, T_3: Cheese with 3% slurry

Table 4: Sensory scores (Mean±Standard Deviation) of control and experimental UF-Ras cheeses during the ripening period

		Treatments			
			UF-Ras cheese with mature cheddar cheese slurry (%)		
Property	Ripening period (days)	Control	1.0	2.0	3.0
Body and texture (40 points)	15	33.80±2.30^{c}	34.80±3.01^{bc}	36.60±1.65^{ab}	37.70±2.00^{a}
	30	34.00±1.63^{b}	35.30±2.87^{b}	37.40±2.01^{a}	38.60±1.65^{a}
	60	34.70±0.95^{b}	35.80±1.55^{b}	38.10±1.45^{a}	38.80±0.92^{a}
	90	35.40±1.07^{c}	37.50±1.18^{b}	38.80±0.79^{a}	39.10±1.37^{a}
Flavor (60 points)	15	4.76±50.20^{c}	2.91±52.60^{bc}	3.22±55.20^{ab}	1.42±56.70^{a}
	30	3.65±51.00^{b}	3.06±53.50^{b}	3.00±56.90^{a}	2.07±57.50^{a}
	60	2.55±51.40^{c}	1.97±54.10^{b}	1.65±57.60^{a}	2.28±57.90^{a}
	90	2.21±53.00^{c}	1.34^{b}±56.30	0.88±58.90^{a}	1.62±59.20^{a}
Total score (100 points)	15	84.00±5.93^{c}	87.40±3.60^{b}	91.80±4.39^{a}	94.40±2.95^{a}
	30	85.00±3.97^{c}	88.80±5.12^{b}	94.30±4.16^{a}	96.10±2.13^{a}
	60	86.10±2.64^{c}	89.90±2.88^{b}	95.70±2.16^{a}	96.70±2.83^{a}
	90	88.40±2.80^{c}	93.80±2.10^{b}	97.70±1.25^{a}	98.30±1.70^{a}

$^{a-c}$Means of the same row with different superscripts differ significantly ($p<0.05$)

intensity that were resembled those of cheddar cheese characteristics as compared with control cheese. So, the positive results of this study support our hypothesis that the addition of mature Cheddar cheese slurry to retentate accelerates the rate of ripening and flavor development in UF-Ras cheese.

CONCLUSION

It could be concluded that, the incorporation of mature Cheddar cheese slurry into retentate can be recommended to improve the overall quality and to accelerate UF-Ras cheese ripening due to its beneficial effects on proteolysis, lipolysis

acceleration and in improving the organoleptic scores of UF-Ras cheese. The best results were obtained by adding mature Cheddar cheese slurry to ultrafiltered cheese milk at a level of 2 or 3%.

SIGNIFICANCE STATEMENT

An attempt has been made to accelerate the ripening speed in UF-Ras cheese via the incorporation of mature Cheddar cheese as a source of enzymes and microorganisms in the retentate. The results indicated that adding mature Cheddar cheese slurry in the retentate during UF-Ras cheese manufacture had a beneficial effect on accelerating UF-Ras cheese ripening and gave best textural and sensory properties.

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