doi: 10.17221/361/2015-CJFS

Effect of Slurry Incorporation into Retentate on Proteolysis of Iranian Ultrafiltered White Cheese

Rahele NEZHAD RAZMJOUI AKHGAR, Javad HESARI and Sodeif AZADMARD DAMIRCHI

Department of Food Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Abstract

Nezhad Razmjoui Akhgar R., Hesari J., Azadmard Damirchi S. (2016): **Effect of slurry incorporation into retentate on proteolysis of Iranian ultrafiltered white cheese**. Czech J. Food Sci., 34: 173–179.

The effect of addition of ripened cheese slurry into retentate on the proteolysis of Iranian ultrafiltered cheese was investigated during 60-day ripening. No significant differences were found in gross composition between experimental samples, however, pH values were significantly higher ($P > 0.05$) in slurry-containing cheese. Urea-polyacrylamide gel of the pH 4.6 insoluble fractions showed that α_{s1}-casein was hydrolysed more rapidly than β-casein. There was no significant difference in the hydrolysis rate of α_{s1}- and β-casein between the two cheeses. The levels of water soluble nitrogen were significantly higher ($P > 0.05$) in slurry-containing samples from day 15. The incorporation of ripened slurry into retentate influenced the peptide profiles obtained by RP-HPLC of the pH 4.6-soluble fractions of the cheeses. The ratio of hydrophilic to hydrophobic peptides was higher in slurry-containing cheese. Total and individual free amino acids were significantly higher ($P > 0.05$) in cheeses containing slurry. It was concluded that the incorporation of ripened cheese slurry into retentate has a beneficial effect on proteolysis acceleration and development of aroma and flavour in Iranian ultrafiltered white cheese.

Keywords: peptide profile; amino acids; rippening; flavour development

Ripening of cheese is a time-consuming process in which well-balanced complex reactions between glycolysis, proteolysis, and lipolysis of the milk components are involved. Proteolysis is the most complex and important event that occurs during the ripening of many cheese varieties and strongly affects the sensory properties of ripened cheeses. The main proteolytic agents of cheese are proteinases of rennet, indigenous milk proteinases, and proteinases and peptidases from starter, non-starter, and secondary starter bacteria (McSweeney 2004). In contrast to traditional cheeses, in which only a small part of the rennet activity added to the milk remained in the curd after the manufacture (Guinee & Wilkinson 1992), in ultrafiltered (UF) white cheeses all the rennet added to retentate is retained in the curd. On the other hand, whey proteins present in UF cheeses at high concentration may inhibit chymosin, microbial rennets (Hayes *et al*. 2002) and probably other proteinases and peptidases. UF cheeses are known to have slower proteolysis and amino acid production during ripening (Hesari *et al.* 2006) and it influences the development of cheese flavour and texture. Several methods exist to accelerate ripening such as enhancing ripening temperatures, application of adjunct cultures, addition of curd slurries/amino acids, to process at high pressure and using exogenous enzymes (Fox *et al.* 1996; Azarnia *et al.* 2006). In general, these methods accelerate proteolysis, however, even though this is relatively easy to achieve, its control is very difficult. The greatest acceleration of ripening using the slurry system was introduced by Kristoffersen *et al.* (1967). Cheddar cheese curd slurried in 3% NaCl to ~40% cheese solids developed full flavour in 4–5 days at 30–35°C when 100 ppm of reduced glutathione were included. Ripening of Cephalotyre 'Ras' cheese was accelerated by incorporation of cheese slurries into cheese milk or curd. Addition of ripened cheese slurry to cheese milk reduced the ripening time of

doi: 10.17221/361/2015-CJFS

the resultant cheese by 25% (Dulley 1976). Addition of pre-ripened full flavour slurry either to pasteurised buffalo cheese milk before adding a starter at a level of 1% or to the curd particles before hooping at a level of 1–2% of cheese milk weight improved the quality or accelerated the flavour development and protein degradation of resultant cheese. Cheese slurries have also been used for the rapid ripening of Brick, Feta, and Swiss cheeses (Fox *et al.* 1996). The objective of the present study was to evaluate the impact of the addition of cheese slurry to the retentate on proteolysis and ripening of Iranian UF white cheese.

MATERIAL AND METHODS

Material. Starter (CHOOZIT™ RA 22 LYO). Freeze-dried concentrated lactic starter for the direct vat inoculation of milk was composed of mesophilic (*Lactobacillus lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis*) and thermophilic (*Streptococcus salivarius* subsp. *thermophilus*) cultures provided by DANISCO (Copenhagen, Denmark). Rennet [Fromase 2200 TL granulate ≥ 2200 (IMCU)/g] as fungal coagulant derived from *Rhizomucor miehei* was obtained from DSM Food Specialities (Seclin, France); reduced glutathione (GSH), sodium citrate, Mn, riboflavin, and cobalt were supplied by Sigma Aldrich (Darmstadt, Germany); raw cow's milk, equipment and filtration moduli were provided by Iran Dairy Industry Inc., Pegah Co. (Urmia, Iran) and West Azarbaijan veterinary laboratory.

Cheese slurry preparation. Cheese slurry was prepared by mixing 500 g of 24-h-old UF cheese with 250 ml of sterile solution of 3% NaCl at 45°C in a blender for 3–4 minutes. GSH, sodium citrate, Mn, riboflavin, and cobalt were also incorporated and the mixture was agitated 30 s daily for 7 days according to the method described by Singh and Kristoffersen (1970).

Cheese making. Experimental UF white cheese samples were made in three trials on three separate days. The retentate was supplied by Iran Dairy Industry Inc., Pegah Co. (Urmia, Iran). Raw milk fat was standardised to 3.5%, and after microfiltration at 50°C under the pressure of 1–2 bar for 40 min and pasteurisation at 74°C for 15 s, it was ultrafiltered at 50°C. The membrane cartridges were the spiral wound type (KMS HpHT™ - HFK™-131; Koch Membrane Systems, Stafford, UK).

Nominal molecular weight cut-off was about 20 kg per mol with a surface area of 15.5 m^2 and the inlet and outlet pressures of the ultrafiltration unit were 5.6 and 1.8 bar, respectively. After pasteurisation of the retentate at 78°C for 60 s, it was cooled to 35°C. Experimental cheeses were prepared, including (1) conventional control samples containing starter (1%) and microbial rennet (28 mg/kg) and (2) in addition to starter and rennet, 25 g/kg slurry incorporated into the retentate. The retentate was filled (400 g) into containers and left to coagulate at 30°C in a coagulation tunnel for 45 minutes. A parchment paper was placed on top of the coagulum in the sealing machine, dry salt (2.5%) was added and containers were sealed with aluminium foil. Cheese packs were kept at 26–28°C for 24 h and then transferred to a cold room (8°C). One cheese of each trial was sampled and analysed at 1, 15, 30, 45, and 60 day of ripening.

Analysis of cheese samples

Gross composition and pH. The cheese samples were analysed for moisture, salt, fat, pH according to Marshall (2005). Total nitrogen (TN) was determined using the Kjeldahl method (IDF 1993).

Nitrogen fractions. Water-soluble nitrogen (WSN) was prepared by a slight modification Kuchroo and Fox (1982) method, as described by Sousa and McSweeney (2001) and the nitrogen content of the fractions was determined by the Kjeldahl method (IDF 1993).

Urea-polyacrylamide gel electrophoresis (urea-PAGE). Urea-PAGE of the pH 4.6-insoluble fraction of the cheeses was performed using a Protean II XI vertical slab-gel unit (Bio-Rad Laboratories Ltd., Watford, UK) according to the method described by Shalabi and Fox (1987).

Total free amino acid content. The total concentration of free amino acids (FAA) in the cheese samples were measured by the trinitrobenzenesulphonic acid (TNBS) assay as described by Kailasapathy (2005).

Individual free amino acid analysis. Concentrations of individual free amino acids were determined according to the method described by Hayaloglu *et al.* (2005), using a Beckman model 6300 amino acid analyser equipped with a Beckman model P-N 338052 Na^+ cation-exchange column (12 × 0.4 cm) (both Beckman Coulter, Fullerton, USA).

RP-HPLC. Peptide profiles of the pH 4.6-soluble fractions of the cheese samples were determined

doi: 10.17221/361/2015-CJFS

by RP-HPLC using a Varian HPLC system (Varian Associates Inc., Walnut Creek, USA) according to the method described by Hayaloglu *et al.* (2005).

Sensory evaluation. Sensory analysis of cheeses after 30 and 60 days of ripening was carried out according to the method described by Di Cagno *et al.* (2011) by 10 experienced panellists from the permanent staff of Pegah Co. (Urmia, Iran). Cheeses were randomly coded and approximately 20 g of cheese samples together with non-salted biscuits and water were presented to panellists. Cheeses were scored from 0 to 10 for qualities that included flavour intensity, aroma intensity, texture, and overall acceptability.

Statistical analysis. The experimental design was split plot based on randomised complete blocks. Significance of differences in results was estimated by one-way ANOVA. The level of significance between treatments was determined at $P < 0.05$. Statistical analysis was performed using SPSS, Version 18 for Windows XP (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Gross composition and pH. Changes in the moisture, salt, fat, protein contents, and pH of the experimental UF cheeses throughout the 60-day ripening are shown in Table 1. Results showed that there were no significant ($P > 0.05$) differences in the main chemical attributes between control and slurry-containing cheeses, suggesting that the addition of additives had a slight influence on gross composition as reported by other researchers for different types of cheeses (Rabie 1989; Karami *et al.* 2009).

However, the cheese containing slurry had significantly higher ($P < 0.05$) pH values than the control cheese from 15 day. The pH of both cheeses decreased until day 45 and increased again on day 60. During ripening, the residual lactose is fermented and the pH value decreases (Guinee *et al.* 2004). Minerals bound to casein micelles, however, result in an increase in the buffering capacity of UF retentate and also change the acidification kinetics of lactic acid bacteria (Mistry & Maubios 2004). Also, during cheese ripening, amino acids were released during the proteolysis reaction, there was a slight increase in the pH value (McSweeney 2004). Similar results were reported by Rabie (1989) and Farag (1987). Perhaps, increased pH at the end of ripening period was due to the production of NH_3 as an effect of the catabolism of released amino acids (Guinee & Fox 2004).

Level of pH 4.6-soluble nitrogen as a percentage of total nitrogen (pH 4.6-SN/TN). The concentrations of pH 4.6-soluble nitrogen (WSN) as a percentage of total nitrogen in experimental UF Iranian white cheeses during ripening are shown in Table 2. In both cheese samples, this index gradually increased during ripening. At the beginning of ripening there were no significant differences between the two cheeses. However, from day 15 the level of pH 4.6-SN/TN in slurry-containing cheese was significantly ($P < 0.05$) higher than that of control cheese. The GSH present in the slurry appeared to have an effect on the rate of bacterial growth and formation of soluble nitrogen (Kristoffersen *et al.* 1967).

Table 1. Chemical composition of Iranian ultrafiltered cheeses throughout 60-day ripening

	Ripening time (day)	Control	Slurry contained
Moisture (%)	1	64.51 ± 0.29[a]	64.27 ± 0.47[a]
	15	64.73 ± 0.35[a]	64.79 ± 0.63[a]
	30	65.24 ± 0.44[a]	65.01 ± 0.32[a]
	45	65.26 ± 1.18[a]	65.03 ± 0.49[a]
	60	65.41 ± 0.56[a]	65.42 ± 0.27[a]
Salt (%)	1	2.24 ± 0.01[a]	2.24 ± 0.01[a]
	15	2.28 ± 0.005[a]	2.28 ± 0.06[a]
	30	2.33 ± 0.005[a]	2.33 ± 0.005[a]
	45	2.43 ± 0.02[a]	2.35 ± 0.10[a]
	60	2.44 ± 0.04[a]	2.40 ± 0.02[a]
Fat (%)	1	14.80 ± 0.10[a]	15.00 ± 0.50[a]
	15	14.80 ± 0.10[a]	14.25 ± 0.25[a]
	30	15.06 ± 0.40[a]	15.03 ± 0.20[a]
	45	15.00 ± 0.00[a]	14.75 ± 0.75[a]
	60	15.03 ± 0.20[a]	15.00 ± 0.00[a]
Protein (%)	1	13.23 ± 0.41[a]	13.30 ± 0.26[a]
	15	13.23 ± 0.35[a]	13.33 ± 0.15[a]
	30	13.53 ± 0.40[a]	13.30 ± 0.32[a]
	45	13.53 ± 0.45[a]	13.63 ± 0.47[a]
	60	13.53 ± 0.11[a]	13.53 ± 0.47[a]
pH	1	4.71 ± 0.04[a]	4.83 ± 0.18[a]
	15	4.58 ± 0.02[b]	4.71 ± 0.03[a]
	30	4.57 ± 0.01[b]	4.68 ± 0.01[a]
	45	4.48 ± 0.01[b]	4.61 ± 0.00[a]
	60	4.62 ± 0.00[b]	4.78 ± 0.06[a]

[a,b]means in the same row followed by different letters are significantly different ($P < 0.05$)

doi: 10.17221/361/2015-CJFS

Table 2. Water soluble nitrogen as a percentage of total nitrogen (SN/TN) in experimental ultrafiltred white cheeses

Age of cheese (day)	Control	Slurry contained
1	4.66 ± 0.47[a]	5.51 ± 0.32[a]
15	5.04 ± 0.02[b]	7.09 ± 0.00[a]
30	5.90 ± 0.10[b]	7.64 ± 0.18[a]
45	6.03 ± 0.31[b]	10.34 ± 0.58[a]
60	6.38 ± 0.17[b]	10.81 ± 0.10[a]

[a,b]means in the same row followed by different letters are significantly different ($P < 0.05$)

In this study, the slurry stimulated the growth of starter bacteria, and as a result enhanced proteolysis, the level of pH 4.6-SN in slurry-containing cheese started to be significantly ($P < 0.05$) higher from day 15 than that of control cheese. As reported by Singh and Kristoffersen (1970), GSH stimulates the formation of active -SH groups. The stimulated formation of active -SH groups in combination with riboflavin, Mn, citrate, and Co suggests an important role of these trace elements in the activity of lactic culture starters. The beneficial effect of agitation on flavour formation in the slurry is attributed in part to the incorporation of oxygen. This is consistent with the findings of other researchers who reported that the addition of slurry into cheese stimulated the formation of soluble nitrogenous compounds, tyrosine, and tryptophan in blue cheese (Rabie 1989) and improved the quality and accelerated the flavour development and soluble nitrogen of Ras cheese.

Urea-PAGE. Urea-PAGE electrophoretograms of the pH 4.6-insoluble fraction of experimental UF white cheeses of trial 1 on day 1, 15, 30, 45, and 60 of the ripening period are shown in Figure 1. The areas of bands formed in urea-PAGE gels were converted to values by Image J software version 4. No differences were observed between electrophoretograms of the two

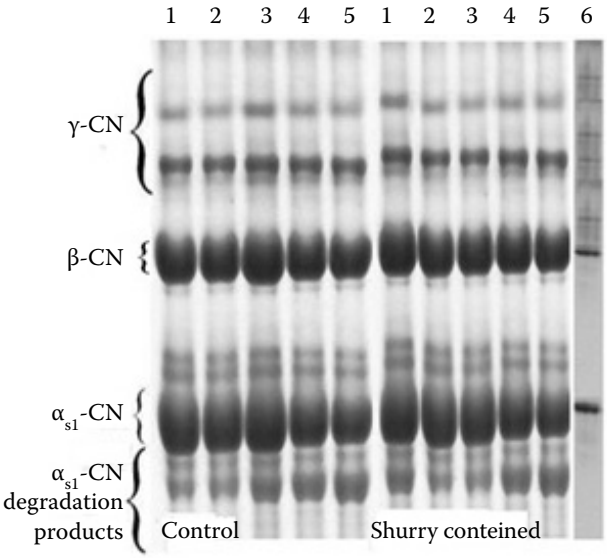

Figure 1. Urea-PAGE electrophoretograms of the pH 4.6-insoluble fractions of experimental Iranian ultrafiltred white cheeses of trial 1 (Lanes 1 to 5 – cheeses after 1, 15, 30, 45, and 60 days of ripening, respectively; lane 6 – sodium caseinate)

cheeses throughout ripening (Table 3). With progression of ripening, degradation of the caseins increased and α_{s1}-casein was hydrolysed to α_{s1}-CN (f24-199). At the end of ripening period, the residual α_{s1}-casein in control and slurry-containing cheeses reached 82.93 and 83.12%, respectively. Singh and Kristoffersen (1971) subjected selected slurries to acrylamide gel electrophoresis to determine α_{s1}-casein hydrolysis. The results indicated that α_{s1}-casein was the only casein that changed significantly during storage and its degradation was similar in slurry and regular cheese.

There was no significant difference in the hydrolysis of β-casein between the two cheeses. Degradation of β-casein was slow throughout ripening; and it is resistant to hydrolysis like in other brined cheeses (Alichanidis *et al.* 1984). The high NaCl concen-

Table 3. Mean values of residual α_{s1}-casein and β-casein in Iranian ultrafiltred white cheeses

Age of cheese (day)	Residual α_{s1}-casein (%)		Residual β-casein (%)	
	control	slurry contained	control	slurry contained
1	100	100	100	100
15	94.23[a]	93.50[a]	97.69[a]	96.58[a]
30	93.62[a]	93.15[a]	96.82[a]	96.26[a]
45	90.76[a]	90.98[a]	93.01[a]	92.39[a]
60	82.93[a]	83.12[a]	90.28[a]	89.66[a]

[a,b]means in the same row followed by different letters are significantly different ($P < 0.05$)

doi: 10.17221/361/2015-CJFS

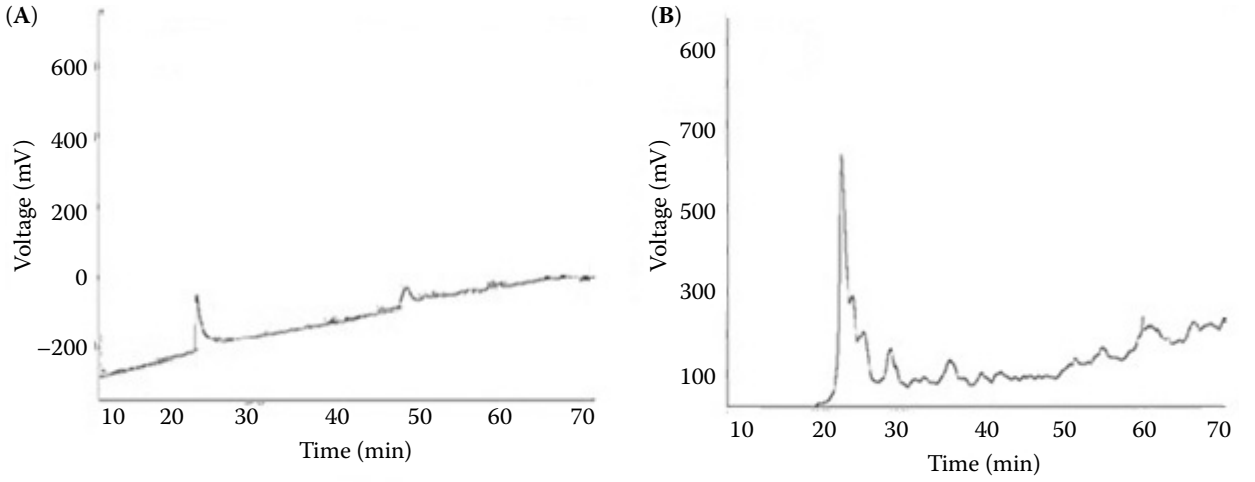

Figure 2. RP-HPLC chromatograms of pH 4.6-soluble fractions of experimental Iranian ultrafiltred white cheeses of trial 1 after 2 months of ripening: (**A**) control and (**B**) containing slurry

tration and low pH of Feta cheese considerably reduced the hydrolysis of β-casein by the coagulant and plasmin, but the degradation of α_{s1}-casein was not inhibited (Alichanidis *et al.* 1984). In white brined cheeses, the major proteolytic agents are the residual coagulant and enzymes from starters or the indigenous microflora. Whey proteins present in UF cheeses at high concentrations may inhibit chymosin, microbial rennets and other proteinases and peptidases (Hesari *et al.* 2006).

RP-HPLC profiles of pH 4.6 soluble fraction. RP-HPLC chromatograms of the pH 4.6-soluble fractions of experimental UF white cheeses of trial 1 after 60 days showed considerable differences in peptide profiles between the two cheeses (Figure 2). In control cheese (Figure 2A), 7 major peaks (with early retention times) and two relative high peaks (with retention times of 23 and 65 min) were observed in the chromatogram. In slurry-containing cheese high concentrations of peptides were in the retention times of 22.5, 28.7, 36, 60, 66, and 68 minutes. Many minor peaks were also observed in the retention time of 22–70 minutes. The peaks observed in the retention time of 22.5–36 min were probably related to low-molecular-weight peptides and free amino acids and those with late retention times indicated hydrophobic peptides. In slurry-containing cheese, additives present in the slurry had a positive effect on the growth of starters and consequently increased their enzymatic activity, on the breakdown of intermediate peptides and production of small peptides and free amino acids. The ratio of hydrophilic to hydrophobic peptides in control and slurry-containing cheeses was 0.85 and 1.94, respectively. Cliffe and Law (1990) made Cheddar cheese using the slurry containing a neutral protease. A high concentration of bitter peptides was detected by RP-HPLC. However, these hydrophobic peptides disappeared considerably when the slurry was prepared in combination with the strain of *Lc. lactis* ssp. *lactis* that was rich in peptidases.

Total and individual free amino acids. The total free amino acid content of slurry-containing cheese was significantly ($P < 0.05$) higher than that of the control sample beginning from day 15 (Table 4). At the end of ripening period, it was twofold higher than that of control cheese due to the stimulation effect of additives present in slurry on the growth of bacteria

Table 4. Total levels of free amino acids during the ripening of experimental ultrafiltred Iranian white cheeses determined by the trinitrobenzenesulphonic acid (TNBS) assay

	Free amino acids (mg glycine/g sample)			
	Day 15	Day 30	Day 45	Day 60
Control	0.10 ± 0.005[b]	0.22 ± 0.041[b]	0.36 ± 0.042[b]	0.54 ± 0.028[b]
Slurry contained	0.40 ± 0.011[a]	0.47 ± 0.028[a]	0.66 ± 0.023[a]	1.10 ± 0.099[a]

[a,b]means in the same row followed by different letters are significantly different ($P < 0.05$)

doi: 10.17221/361/2015-CJFS

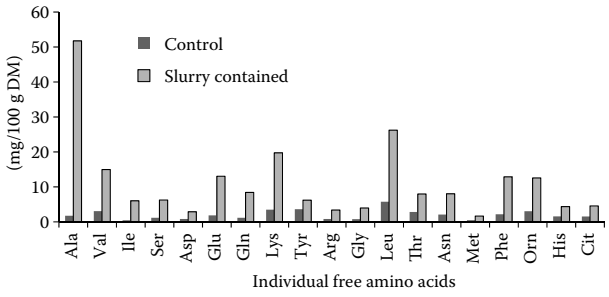

Figure 3. Concentrations (mg/100 g DM) of individual free amino acids in experimental ultrafiltred Iranian white cheeses of trial 1 after 2 months of ripening

(Kristoffersen *et al.* 1967) that degraded intermediate peptides to free amino acids and sapid compounds.

Figure 3 shows the concentrations of individual free amino acids in experimental Iranian UF white 60-days-old cheeses of trial 1. Levels of all individual amino acids were also higher in slurry-containing cheeses than in control samples. In control cheeses a dominant free amino acid was leucine, and to a lesser extent valine, lysine, and tyrosine, while in slurry-containing cheeses dominant free amino acids were alanine, leucine, lysine, and valine at higher concentrations than those of control cheese.

The liberation of amino acids is carried out by peptidases of starter bacteria. However, different starters produce different concentrations of individual free amino acids on the basis of their enzymatic system and the degree of autolysis in the cheese. Other research groups (Alichanidis *et al.* 1984; Michaelidou, *et al.* 2003) reported that leucine, gluthamine, valine, and lysine were principle free amino acids in Feta cheese made from cows' milk.

Sensory evaluation. The mean sensory scores of the experimental cheeses on day 60 are shown in Table 5. Sensory scores on day 30 were similar and are not shown. Significant differences ($P < 0.05$) were noted between the two cheeses in terms of all sensory scores except texture. Slurry-containing cheese received significantly ($P < 0.05$) higher scores for flavour, aroma and overall acceptability compared to control cheese, indicating that the incorporation of slurry into retentate resulted in improvement in flavour and aroma development. The lower level of flavour and aroma intensity in control cheese may be correlated with lower levels of nitrogen fractions and free amino acids as reported by other researchers (Madkor *et al.* 2000; Hayaloglu *et al.* 2005). The score for texture did not significantly ($P < 0.05$) vary between the control and slurry-containing cheeses.

Table 5. Sensory scores at the end of ripening of experimental ultrafiltred white cheeses; control and containing slurry

	Control	Slurry contained
Flavour	4.66 ± 0.57[b]	8.33 ± 0.57[a]
Aroma	4.33 ± 0.57[b]	8.50 ± 0.57[a]
Texture	4.33 ± 0.57[a]	5.00 ± 1.00[a]
Overall acceptability	5.33 ± 0.57[b]	8.16 ± 0.28[a]

[a,b]means in the same row followed by different letters are significantly different ($P < 0.05$)

CONCLUSION

This research investigated the effect of ripened cheese slurry addition on proteolysis and accelerated ripening of Iranian UF white cheese. Results of this study indicated that the additives present in slurry had a stimulation role in the activity of lactic culture starters and their enzymes and consequently their contribution in secondary proteolysis. The incorporation of ripened cheese slurry into retentate seems to be a beneficial method for accelerating secondary proteolysis, development of flavour, aroma and formation of sapid compounds in UF cheeses that ripen more slowly than traditional cheeses.

Acknowledgements. The authors are grateful to Iran Dairy Industry Inc., Pegah Co. (Urmia) and West Azarbaijan Veterinary Office for technical support in cheese making and analysing.

References

Alichanidis E., Anifantakis E.M., Polychroniadou A., Nanou M. (1984): Suitability of some microbial coagulants for Feta cheese manufacture. Journal of Dairy Research, 51: 141–147.

Azarnia S., Robert N., Lee B.H. (2006): Biotechnological methods to accelerate Cheddar cheese ripening. Critical Review of Biotechnology, 26: 121–143.

Cliffe A.J., Law B.A. (1990): Peptide composition of enzyme-treated Cheddar cheese slurries, determined by reverse phase high performance liquid chromatography. Food Chemistry, 36: 73.

Di Cagno R., De Pasquale I., De Angelis M., Buchin S., Calasso M., Fox P.F., Gobbetti M. (2011): Manufacture of Italian Caciotta-type cheeses with adjuncts and at-

doi: 10.17221/361/2015-CJFS

tenuated adjuncts of selected non-starter lactobacilli. International Dairy Journal, 21: 245–260.

Dulley J.R. (1976): The utilization of cheese slurries to accelerate the ripening of Cheddar cheese. Australian Journal of Dairy Technology, 31: 143–148.

Farag A. (1987): Chemical and technological studies on blue cheese made from recombined milk. [PhD. Thesis.] Zagazig University, Zagazig, Egypt.

Fox P.F., Wallace J.M., Morgan S., Lynch C.M., Niland E.J., Tobin J. (1996): Acceleration of cheese ripening. Antonie Van Leeuwenhoek, 70: 271–297.

Guinee T.P., Fox P.F. (2004): Salt in cheese: physical, chemical and biological aspects. In Fox P.F.: Cheese: Chemistry, Physics and Microbiology, General Aspects. Vol. 1. 3rd Ed. London, Chapman and Hall: 207–259.

Guinee T.P., Wilkinson M.G. (1992): Rennet coagulation and coagulants in cheese manufacture. The Society of Dairy Technology, 45: 94–104.

Hayaloglu A.A., Guven M., Fox P.F., McSweeney P.L.H. (2005): Influences of starters on chemical, and sensory changes in Turkish White-brined cheese during ripening. Journal of Dairy Science, 88: 3460–3474.

Hayes M.G., McSweeney P.L.H., Kelly A.L. (2002): The influence of native and heat denatured whey proteins on enzyme activity. 2. Chymosin. Milchwissenschaft, 57: 264–267.

Hesari J., Ehsani M.R., Khosroshahi A., McSweeney P.L.H. (2006): Contribution of rennet and starter to proteolysis in Iranian UF white cheese. Lait, 86: 291–302.

IDF (1993): Determination of Nitrogen Content, Standard 20B. Brussels, International Dairy Federation.

Kailasapathy K., Lam S.H. (2005): Application of encapsulated enzymes to accelerate cheese ripening. International Dairy Journal, 15: 929–939.

Karami M., Ehsani M.R., Mousavi S.M., Rezaei K., Safari M. (2009): Microstructural properties of fat during the accelerated ripening of ultrafiltered-Feta cheese. Food Chemistry, 113: 424–434.

Kristoffersen T., Mikolajcik E.M., Gould I.A. (1967): Cheddar cheese flavor. IV. Directed and accelerated ripening process. Journal of Dairy Science, 50: 292–297.

Kuchroo C.N., Fox P.F. (1982): Soluble nitrogen in cheddar cheese: Comparison of extraction procedures. Milchwissenschaft, 37: 331–335.

Madkor S.A., Tong P.S., El Soda M. (2000): Ripening of Cheddar cheese with added attenuated adjunct cultures of lactobacilli. Journal of Dairy Science, 83: 1684–1691.

Marshall T.R. (2005): Standard Methods for the Examination of Dairy Products. Washington, DC, American Public Health Association.

McSweeney P.L.H. (2004): Biochemistry of cheese ripening: Introduction and overview in cheese: In: Fox P.F.: Cheese: Chemistry, Physics and Microbiology, General Aspects. Vol. 1. 3rd Ed. London: Chapman and Hall: 347–360.

Michaelidou A., Katsiari M.C., Kondly E., Voutsinas L.P., Alichanidis E. (2003): Effect of a commercial adjunct culture on proteolysis in low-fat Feta-type cheese. International Dairy Journal, 13: 179–189.

Mistry V.V., Maubios J.L. (2004): Application of membrane separation technology to cheese production. In: Fox P.F.: Cheese: Chemistry, Physics and Microbiology, General Aspects. Vol. 1. 3rd Ed. London, Chapman and Hall: 261–285.

Rabie A.M. (1989): Acceleration of blue cheese ripening by cheese slurry and extracellular enzymes of *Penicillium roqueforti*. Lait, 69: 305–314.

Shalabi S.I., Fox P.F. (1987): Electrophoretic analysis of cheese, comparison of methods. Irish Journal of Food Science and Technology, 11: 135–151.

Singh S., Kristoffersen T. (1971): Cheese flavor development using direct acidified curd. Journal of Dairy Science, 55: 744–749.

Singh S., Kristoffersen T. (1970): Factors affecting flavor development in cheddar cheese slurries. Journal of Dairy Science, 53: 533–536.

Sousa M.J., McSweeney P.L.H. (2001): Studies on the ripening of Cooleeney, an Irish farmhouse Camembert-type cheese. Irish Journal of Agriculture and Food Research, 40: 83–95.

Received: 2015–07–15
Accepted after corrections: 2016–02–09

Corresponding author:

Rahele Nezhad Razmjoui Akhgar, University of Tabriz, Faculty of Agriculture, Department of Food Science and Technology, Tabriz, 29 Bahman Blvd, Iran; E-mail: razmjooi@yahoo.com