academicJournals

Vol. 12(37), pp. 5588-5594, 11 September, 2013
DOI: 10.5897/AJB2013.12912
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of coagulating enzyme types (commercial calf rennet, *Aspergillus niger* var. *awamori* as recombinant chymosin and *rhizomucor miehei* as microbial rennet) on the chemical and sensory characteristics of white pickled cheese

Fatma Çepoğlu[1] and Mutlu B. Güler-Akın[2]*

[1]Commodity Exchanges, Şanlıurfa, Turkey.
[2]Department of Food Engineering, Faculty of Agriculture, Harran University, Şanlıurfa, Turkey.

Accepted 26 August, 2013

The possibilities of using recombinant chymosin as an alternative coagulant to commercial calf rennet in the production of white pickled cheese was investigated. For this purpose, white pickled cheese produced by using commercial calf rennet, recombinant chymosin (*Aspergillus niger* var. *awamori*) and microbial rennet (*Rhizomucor miehei*) were compared in terms of their chemical and organoleptic properties. The cheese samples were stored in brine containing 12% salt at 4±1°C for 60 days. In the study, which was carried out in duplicate, pH, titratable acidity, dry matter, fat, fat-in-dry matter, protein, salt, nitrogeneous compounds (water sobuble nitrogen (WSN), ripening index (RI), non-protein nitrogen (NPN), electrophoretic and organoleptic properties of the cheese samples were determined at 1, 15, 30 and 60 days of storage. According to the results, the effects of enzyme type on the titratable acidity, dry matter, salt, nitrogeneous compounds and all sensory properties, except for the odour was significant ($p<0.05$). At the end of storage, the titratable acidity, salt, WSN, RI, NPN values and sensory scores of the cheeses increased, while the pH, fat, total nitrogen (TN), protein, β- and α_{s1}-casein contents of cheeses decreased compared to initial values.

Key words: White pickled cheese, calf rennet, recombinant chymosin, microbial rennet.

INTRODUCTION

Milk-clotting enzymes are the primary active agents in cheesemaking; coagulation of milk is a crucial step, which involves the enzyme-mediated cleavage of κ-casein at the peptide bond Phe 105-Met 106 that renders the casein micelles unstable, and evantually causes aggregation that yields a clot and a gel afterwards (Fox and McSweeney, 1999; Silva et al., 2003). In addition to such a specific proteolytic activity, milk clotting enzymes usually possess a broader proteolytic activity towards α_s- and β-caseins, which eventually aids ripening. Commercial calf rennet consists mainly of two proeolytic enzymes, namely chymosin (EC 3.4.23.4) and pepsin.

*Corresponding author. E-mail: mutluakin@harran.edu.tr.

Abbreviations: WSN, Water sobuble nitrogen; **NPN,** non-protein nitrogen; **PPN,** proteose-pepton nitrogen; **RI,** ripening index; **EDTA,** ethylenediaminetetra acetate; **FDM,** fat-in-dry matter; **TN,** total nitrogen.

Chymosin, extracted from abomasa of sucking calves (calf rennet), was the first and still is the most widely used milk-clotting enzyme in traditional cheese-making world-wide (Fox, 1987; Guinee and Wilkinson, 1992; Fox and McSweeney, 1999, Feng et al., 2011). Rennet is used in cheese manufacturing primarily as a milk coagulant. Other enzymes present in rennet also play an important role in cheese production, especially in cheese ripening and may be a cause of the development of bitterness during storage (Rogelj et al., 2001). However, in recent decades, due to a shortage of calf rennet on world markets, alternative milk-clotting enzymes of different origins have been investigated.

The most common rennet substitutes include bovine, porcine and, to a lesser extent, chicken pepsins and microbial proteases from *Mucor miehei*, *Mucor pucillus* and *Cryphonectria parasitica* (formerly *Endothia parasitica*). Proteolytic enzymes of fungal origin have received considerable attention; especially extracellular enzymes of *C. parasitica*, *M. miehei* and *M. pucillus* have received wider acceptability on the industrial scale due to their high milk-clotting and low proteolytic activities (Şeker et al., 1998). Most of the plant coagulants have very high proteolytic capacity and, therefore, they are largely not suitable for cheese-making.

More recently, the gene for chymosin has been cloned and inserted into micro-organisms such as *Escherichia coli* (Chy-Max, Pfizer), *Kluyveromyces lactis* (Maxiren, Gist-Brocades, Delft-Holland) and *Aspergillus niger* var. *awamori* (Chymogen, Genencor) and yeast, resulting in the expression of chymosin for use as a coagulant. The enzymic properties of this biotechnologically derived chymosin are indistinguishable from those of the native calf chymosin, while in cheese manufacture, there are no major differences between cheeses made with the non-animal or calf chymosins (Guinee and Wilkinson, 1992; Fox and McSweeney, 1999; Kosikowski and Mistry, 1997, Broome and Limsowtin, 1998). In addition to the benefit that such chymosin can be produced in large-scale fermentors at low cost, recombinant, highly pure chymosin has also some other advantages such as specific, low proteolytic activity, predictable coagulation behavior, kosher certification, and vegetarian approval (Repelius, 1998; Rogelj et al., 2001).

The aim of this research was to make a comparison between Turkish white brined cheeses manufactured from cow's milk by using alternative coagulating enzyme to commercial and microbial rennets. For this purpose, recombinant chymosin was employed as an alternative coagulant in white pickled cheese production.

MATERIALS AND METHODS

Cow's milk (morning milking) used in the manufacture of white pickled cheese was collected three times during March 2004 from Holstein cattle in Şanlıurfa. In this study, Rennilase 150 L Type t (from *Rhizomucor miehei* by deep fermentation) and Chy-Max 15 T Plus (from *A. niger* var. *Awamori* by recombinant DNA technology, 100% chymosin) were used as alternative coagulating enzymes. The liquid commercial rennet (90% chymosin+10% pepsin) and alternative coagulating enzymes were supplied by Peyma-Chr. Hansen, Gayrettepe-İstanbul, Turkey. The milks were inoculated with DVS mesophilic homofermentative culture (R-703) consisting of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* (obtained from Peyma-Chr. Hansen, Gayrettepe, Turkey).

Production of cheese

Cheese was produced at the pilot dairy plant of the Food Engineering Department, Harran University. Two different trials were performed for the manufacture of cheese. In each trial, the large flocks were removed from raw cow's milk using a cloth filter. The milk was heat-treated to 72°C for 2 min, cooled to 32°C, inoculated with starter culture (1% inoculum) and $CaCl_2$ was added at a rate of 0.02%. After fermentation for 30 min, the milk was divided into three equal portions (30 L each). The first batch (A) was coagulated with liquid commercial rennet (strength, 1: 8000), the second batch (B) was coagulated with Chy-Max (strength, 1: 15000) and third batch (C) was coagulated with Rennilase (strength, 1: 50000). Following coagulation, the coagulum was cut with cutting harps about 1 cm^3 size and drained. The curds were transferred into batchs lined with cheese cloth to drain whey and pressed for about 3.5 h at 20±2°C. Then, curd was cut into 7x11x11cm segments to shape and these shaped curds were put into pre-brined solution containing 15% NaCl (w/v) at 18°C for 5 h. Following pre-brining, the cheeses were packaged in plastic cups (1 kg) containing brine (concentration 12%) and transferred to cold storage (4+1°C) for 60 days.

Analytical methods

The pH of the milk (TSE, 1994) and cheeses (TSE, 1995) was measured using a digital pH-meter (model of Orion 250 A, Orion Research Inc., Boston, USA). The protein content of the milk and cheeses were determined by the Kjeldahl method (Gripon et al., 1975). The total fat and dry matter contents of the cheese samples were determined by the Gerber (TSE, 1994), and gravimetric methods (AOAC, 1990), respectively. Salt content of the cheese was determined by Mohr titration method (Anonymous, 1978) water sobuble nitrogen (WSN), non-protein nitrogen (NPN) and proteose-pepton nitrogen (PPN) contents were determined according to Gripon et al. (1975). Ripening index (RI) was estimated by using the formula: (WSN/TN)x 100, as proposed by Alais (1984).

Products of proteolysisi in the cheese samples were analyzed by mini urea polyacrylamide gel electrophoresis (Urea-PAGE). Electrophoresis was carried out on a vertical slab unit (Bio-Rad Laboratories, Inc. 1000 Alfred Nobel Drive, Herculus, California, USA) and the stacking gel system described by Creamer (1991). Samples were prepared by gratting 0.5 g of each cheese into 25 ml of sample buffer (0.092 g ethylenediaminetetra acetate (EDTA), 1.08 G Tris, 0.55 g boric acid and 36 g urea made up to 100 ml and adjusted to pH 8.4). Each sample was centrifuged at 10000 *g* for 10 min and 2 ml from the middle portion was taken. All samples were mixed with 3% each of 0.1% (w/v) bromphenol blue solution and mercaptoethanol. 5 µl of the 2% cheese solutions were used for electrophoresis. For dying the gels Coosmassie Blue R-250 dye solution was used.

The samples were organoleptically assessed by ten panelists using a sensory rating scale of 1-35 for flavour and taste, 1-35 for consistency, 1-20 for odour and 1-20 for color and appearance as described by TSE (1995). The panel of assessors was an external panel of non-smokers who were very familiar with brined cheese

Table 1. Physio-chemical properties of Turkish white cheeses produced by using different coagulating enzymes.

Property	Cheese*	Storage period (day)			
		1	15	30	60
pH	A	5.55±0.205^{b3}	5.90±0.042^{b1}	5.92±0.021^{b1}	5.62±0.226^{b2}
	B	5.59±0.057^{ab3}	6.08±0.106^{ab1}	5.97±0.000^{ab1}	5.65±0.007^{ab2}
	C	5.61±0.049^{a3}	5.96±0.042^{a1}	5.99±0.092^{a1}	5.88±0.042^{a2}
Titratable acidity (% lactic acid)	A	0.565±0.021^{a1}	0.465±0.007^{a2}	0.450±0.000^{a2}	0.620±0.014^{a1}
	B	0.515±0.021^{c1}	0.393±0.004^{c2}	0.405±0.007^{c2}	0.540±0.000^{c1}
	C	0.525±0.021^{b1}	0.435±0.035^{b2}	0.425±0.035^{b2}	0.560±0.014^{b1}
Dry matter (%)	A	44.08±0.608^{ab1}	43.24±0.735^{ab2}	42.09±0.665^{ab3}	41.87±0.417^{ab3}
	B	43.37±0.255^{b1}	42.54±0.141^{b2}	41.74±0.191^{b3}	41.88±0.304^{b3}
	C	44.62±0.163^{a1}	43.65±0.184^{a2}	42.54±0.438^{a3}	42.26±0.325^{a3}
Fat (%)	A	21.00±0.000^{a1}	21.25±0.354^{a1}	20.00±0.000^{a2}	20.00±0.000^{a2}
	B	21.00±0.700^{a1}	20.63±0.177^{a1}	20.25±0.354^{a2}	20.25±0.354^{a2}
	C	21.25±1.061a^{1}	21.25±1.061^{a1}	19.75±1.061^{a2}	19.5±0.707^{a2}
Fat in dry matter	A	47.65±0.658^{a1}	49.15±0.021^{b1}	47.52±0.750^{b2}	47.78±0.474^{b2}
	B	48.42±1.351^{b1}	48.49±0.573^{a1}	48.53±1.060^{c2}	48.37±1.195^{c2}
	C	47.63±2.199^{a1}	48.69±2.630^{a1}	46.44±2.970^{a2}	46.16±2.029^{a2}
Protein (%)	A	18.65±0.948^{1}	17.21±0.912^{2}	16.95±0.884^{2}	16.55±0.679^{2}
	B	17.99±0.410^{1}	17.08±0.120^{2}	16.65±0.389^{2}	16.47±0.594^{2}
	C	18.86±0.940^{1}	17.46±1.365^{2}	17.59±1.407^{2}	17.29±1.110^{2}
Salt (%)	A	3.13±0.078^{b3}	3.61±0.240^{b2}	3.84±0.318^{b2}	4.14±0.205^{b1}
	B	3.46±0.014^{a3}	3.86±0.085^{a2}	3.99±0.042^{a2}	4.26±0.042^{a1}
	C	3.33±0.127^{a3}	3.94±0.042^{a2}	4.07±0.035^{a2}	4.32±0.106^{a1}

*A, Manufactured by using commercial liquid rennet; B, manufactured by using recombinant chymosin (*A. niger* var. *awamori*); C, manufactured by using Rennilase (*R. miehei*); **Different letters in the same column indicate significant differences among the samples depending on enzyme type, and different numbers in the same line indicate significant differences among the samples depending on storage time ($p<0.05$).

and were checked on the basis of sensory acuity and consistency. The experiment was designed according to a 3x4 factorial design. The data were analysed statistically by means of SPSS statistical software program (version 5.0). Statistically different groups were determined by the least significant difference (LSD) test (Bek and Efe, 1995).

RESULTS AND DISCUSSION

Physico-chemical characteristics

The chemical composition of the cow's milk used to produce the cheese (data not shown) fell within the following averages (n=2): titratable acidity 0.17 (±0.013)% lactic acid, pH 6.64 (±0.071), dry matter 12.59 (±0.156)%, fat 3.20 (±0.141)%, protein 3.91 (±0.106)% and lactose 4.73 (±0.04)%. Variation in physio-chemical properties of the white cheeses throughout ripening period are shown in Table 1.

The data reveals that the coagulating enzyme type had significant effect on titratable acidity, dry matter and salt content in cheese throughout the ripening time at 4°C. At the begining of storage, the titratable acidity value in the cheese A was the highest. Titratable acidity of samples decreased during storage up to day 30, and then increased; this trend could be associated with diffusion of lactic acid from the cheese into the brine. Similar results were reported by Saldamlı and Kaytanlı (1998) and Irigoyen et al. (2001).

The pH values of the ripened cheeses ranged between 5.62 to 5.88. The trends in the variation of pH values over the ripening period were similar for all batches. In all cheese batches, the pH increased until day 30 of ripening, after which it decreased slightly. Similar finding were reported earlier (Irigoyen et al., 2001) and could be attributed to the breakdown of lactic acid when all the

Table 2. Nitrogen fractions of Turkish white cheeses produced by using different coagulating enzymes (%).

Property	Cheese*	Storage period (day)			
		1	15	30	60
Total nitrogen	A	2.923±0.148[1]	2.696±0.143[2]	2.656±0.139[2]	2.594±0.107[2]
	B	2.820±0.064[1]	2.677±0.019[2]	2.609±0.061[2]	2.582±0.093[2]
	C	2.956±0.148[1]	2.736±0.214[2]	2.756±0.221[2]	2.709±0.174[2]
Water soluble nitrogen (%)	A	0.325±0.016[b1]	0.337±0.015[b2]	0.345±0.016[b3]	0.374±0.020[b4]
	B	0.308±0.014[c1]	0.311±0.016[c2]	0.323±0.015[c3]	0.360±0.014[c4]
	C	0.348±0.017[a1]	0.363±0.022[a2]	0.373±0.024[a3]	0.411±0.032[a4]
Ripening index (%)	A	11.11±0.007[b1]	12.49±0.106[b2]	12.98±0.064[b3]	14.42±0.170[b4]
	B	10.92±0.255[c1]	11.62±0.495[c2]	12.36±0.283[c3]	13.95±0.049[c4]
	C	11.78±0.021[a1]	13.26±0.233[a2]	13.54±0.212[a3]	15.15±0.205[a4]
Non-protein nitrogen	A	0.163±0.008[b1]	0.171±0.004[b2]	0.211±0.013[b3]	0.234±0.019[b4]
	B	0.144±0.007[c1]	0.151±0.006[c2]	0.180±0.004[c3]	0.189±0.008[c4]
	C	0.182±0.005[a1]	0.192±0.011[a2]	0.223±0.009[a3]	0.237±0.007[a4]

*A, Manufactured by using commercial liquid rennet; B, manufactured by using recombinant chymosin (*A. niger* var. *awamori*); C, manufactured by using Rennilase (*R. miehei*); **Different letters in the same column indicate significant differences among the samples depending on enzyme type, and different numbers in the same line indicate significant differences among the samples depending on storage time ($p<0.05$).

residual lactose has been metabolized and the production of basic substances is completed (McSweeney and Fox, 1993), and to the metabolization of the non-protein nitrogen components and organic acids (Farkye and Fox, 1990). Salt content of the cheese A was lower than the other cheeses. Salt contents in the experimental cheeses increased during the ripening period because of diffusion of salt from the surfaces into the centre of the cheese where samples were taken for these determinations.

Total protein content of the cheeses decreased slightly over the ripening period, with values being comparable to the results previously reported for white pickled cheese (Saldamlı and Kaytanlı, 1998). The fat content remained steady in all cheese batches throughout the ripening period. In addition, the moisture and fat-in-dry matter (FDM) contents of all ripened cheeses met the figures given by the Turkish Standardization Institute (TSE) for the first quality white pickled cheese (TSE, 1995). Dry matter contents in the cheese B were lower than cheeses A and C. It could be deduced that the recombinant chymosin made a structure with the milk components that retained more liquid during the wheying stage of the cheese. Dry matter contents in white cheeses decreased during the ripening period because of new occuring ionic group as a result of breaking the peptide bond (Creamer and Olson, 1982) and also increasing water binding capacity of protein in cheese which is stored at low temperature (Salama et al., 1982). These results are in agreement with those reported by Saldamlı and Kaytanlı (1998) and indicated that the cheeses A, B and C did not significantly differ ($p>0.05$) in fat, fat-in-dry matter and protein content throughout ripening. Also, the coagulating enzymes did not affect pH, protein, fat or fat in dry matter contents of white cheeses significantly ($p>0.05$).

Nitrogen fractions

The WSN values increased throughout ripening in all cheese samples. The different milk-clotting enzymes employed yielded significant differences in the WSN values (Table 2); the cheese made with the *R. miehei* (cheese C) had higher WSN values. The WSN values were similar to the values recorded for the same cheese variety by Saldamlı and Kaytanlı (1998). Ripening index (WSN/total nitrogen (TN)) values (ripening index) increased throughout ripening in all cheese samples, however, RI levels were significantly higher ($p<0.05$) in the cheese C. The formation of water soluble nitrogen compounds during ripening is an indicator of casein hydrolysis brought about by the action of the rennet and the milk proteases present at the start of ripening (Irigoyen et al., 2001). Therefore, WSN value is an index of the rate and extent of proteolysis. The NPN values increased continuously over the ripening period in all cheeses (Table 2). The cheeses made by using *A. niger* var. *awamori* (cheese B) showed lower NPN values.

Electrophoresis of the caseins

The α- and β-casein underwent a significant decrease

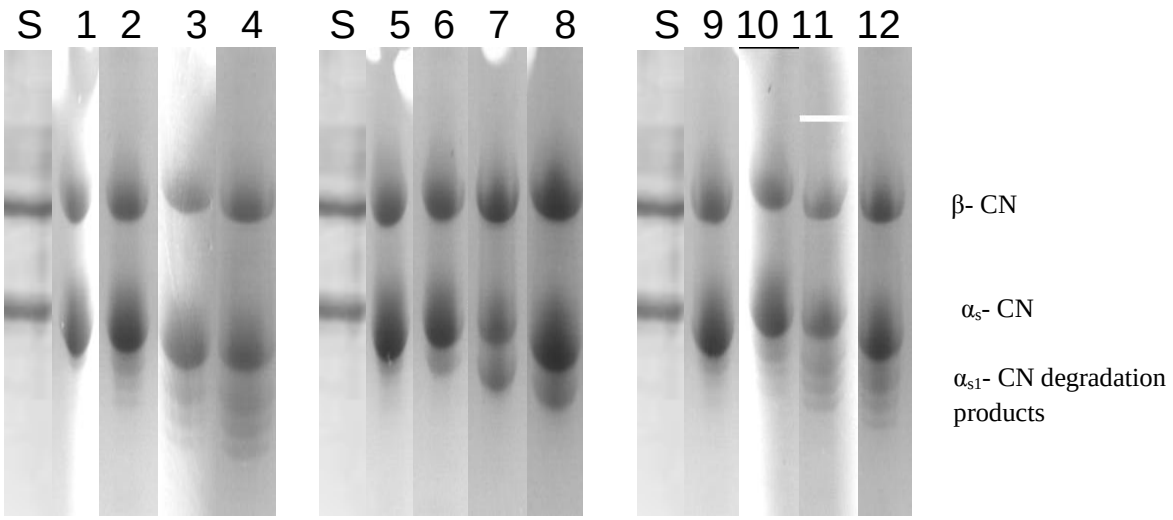

Figure 1. Urea-PAGE electrophoretograms of bovine sodium caseinate and white pickled cheese manufactured using commercial calf rennet (lanes 1-4), recombinant chymosin (lanes 5-8) and microbial rennet (lanes 9-12) after 1, 15, 30 and 60 day of ripening.

during ripening (Figure 1). This was a result of the action of the residual rennet in combination with the action of the hydrolytic enzymes of the microorganisms present in the cheeses (Irigoyen et.al., 2001). The first casein fraction to be broken down was the α_{s1}-casein and α_{s1}-casein degradation products in all cheese samples at different rates. The influence of the milk-clotting enzyme on the α_s-casein was more pronounced. This finding was in agreement with the results reported by Saldamlı and Kaytanlı (1998), who employed capillary electrphoresis to study the differential degradation of α_s-casein by various coagulants. At the end of the ripening period, the bonds representing residual α_{s1}-casein and α_{s1}-casein degradation products in the cheese made using *A. niger* var. *awamori* (cheese B) were less visible than that of the cheeses A and C. The α_{s1}-casein fraction is the fraction most intensely broken down by rennet proteases during ripening (Farkye and Fox, 1990).

Leu190-Tyr191 and Ala187-Phe188 are the regions on the β-casein that are most susceptible to the action of chymosin (Whyte, 1995). Breakdown of the β-caseins was less pronounced than that of the α_s-caseins in both the experimental cheese batches. Several other workers have previously reported greater resistance of β-caseins to enzymatic hydrolysis (Fox and Law, 1991). In this study, the degree of β-casein degradation differed between the coagulating enzymes tested. At the end of the ripening period, the residual β-casein in the cheese B was more visible than the cheeses A and B.

Sensory analysis

Table 3 shows the sensory attributes evaluated by the panel group for each cheese samples. The cheese B (made by using *A. niger* var. *awamori*) recieved highest scores for colour and appearance, odour and flavour at the end of storage. Total sensory scores (colour and appearance, body and texture, odour and flavour) of the cheese B were close to the cheese C and both had higher sensory scores than the cheese A. During storage, total sensory scores of all the cheeses increased. The results of the sensory analysis plainly showed the appreciable influence of ripening time, with the cheeses becoming more moist and tender with higher adherence and characteristic texture scores after 60 days.

Conclusions

Coagulating enzyme types and storage period had significant effects on the titratable acidity, dry matter and the sensory characteristics of the cheeses. The titratable acidity and dry matter content of the cheeses made by using *A. niger* var. *awamori* (cheese B) were lower than the other cheeses and they received the highest sensory scores from the panelists.

The bonds corresponding to nitrogen fractions were less visible in the cheese B; and proteolytic activity on the β and α_{s1}-caseins, especially on the α_{s1}-caseins, was likewise lower. Thus, both the formation and the subsequent degradation of the α_{s1}-casein took place less rapidly in the cheeses made by using *A. niger* var. *awamori* (cheese B). According to the results obtained, it could be speculated that the overall quality of the cheese made with *A. niger* var. *awamori* (cheese B) was quite similar to the the cheeses made with commercial calf rennet or microbial rennet. The cheese B had the lowest

Table 3. Sensory characteristics of Turkish white cheeses produced by using different coagulating enzymes (%).

Property	Cheese*	Storage period (day)			
		1	15	30	60
Color and appearance	A	19.28±0.014^{b1}	18.92±0.007^{b12}	19.06±0.276^{b2}	19.14±0.191^{b12}
	B	18.91±0.134^{a1}	19.79±0.297^{a12}	19.32±0.332^{a2}	19.53±0.106^{a12}
	C	20.35±0.205^{a1}	19.27±0.262^{a12}	18.97±0.191^{a2}	19.28±0.113^{a12}
Body and texture	A	28.45±0.318^{b2}	32.81±0.205^{b1}	32.90±0.255^{b1}	32.90±0.141^{b1}
	B	30.47±0.757^{b2}	33.47±0.156^{b1}	31.64±0.396^{b1}	32.43±0.099^{b1}
	C	33.58±0.339^{a2}	31.69±0.339^{a1}	33.66±0.219^{a1}	33.28±0.247^{a1}
Odour	A	9.28±0.361^{2}	9.70±0.170^{1}	9.52±0.092^{12}	8.67±0.042^{3}
	B	9.27±0.000^{2}	9.69±0.198^{1}	9.44±0.021^{12}	8.80±0.283^{3}
	C	9.470±0.283^{2}	9.53±0.035^{1}	9.435±0.021^{12}	8.68±0.318^{3}
Flavour	A	29.28±0.071^{b3}	29.26±0.014^{b3}	30.78±0.078^{b1}	31.25±0.219^{b2}
	B	31.61±0.198^{a3}	31.64±0.269^{a3}	32.36±0.396^{a1}	31.28±0.389^{a2}
	C	28.77±0.438^{b3}	29.28±0.389^{b3}	31.39±0.042^{b1}	30.40±0.566^{b2}
Total score	A	86.28±0.042^{b3}	90.66±0.354^{b2}	92.25±0.700^{b1}	91.95±0.071^{b2}
	B	90.25±1.089^{a3}	94.59±0.325^{a2}	92.75±0.354^{a1}	92.03±0.467^{a2}
	C	92.71±0.071^{a3}	89.76±0.346^{a2}	93.45±0.389^{a1}	91.63±0.523^{a2}

*A, Manufactured by using commercial liquid rennet; B, manufactured by using recombinant chymosin (*A. niger* var. *awamori*); C, manufactured by using Rennilase (*R. miehei*). **Different letters in the same column indicate significant differences among the samples depending on enzyme type, and different numbers in the same line indicate significant differences among the samples depending on storage time ($p<0.05$).

level of proteolysis and recieved higher sensory scores than the other cheeses during storage. Consequently, it is thought that recombinant chymosin can be successfully used in white pickled cheese production as an alternative coagulant to the commercial calf rennet and microbial rennet. Therefore, this would allow the cheese consumption by consumers who object to use of animal-derived products in cheese for religious, moral and dietry reasons.

ACKNOWLEDMENT

This study was financially supported by the Research Fund of Harran University (Project No: 411) and extracted from Ms. thesis of Fatma Çepoğlu.

REFERENCES

Alais C (1984). Science du Lait. 4. Edition, Edition Sepaic, Paris, France.

Anonymous (1978). Cheese, cheese products and fermented milk. University of Reading, Food Science and Technology Department, Reading, England.

AOAC (1990). Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Bek Y, Efe E (1995). Research and Evaluation Methods. University of Cukurova Faculty of Agriculture Lecture Notes No:71, Adana, Turkey.

Broome MC, Limsowtin GKY (1998). Milk Coagulants. Aust. J. Dairy Technol. 53:188-190.

Creamer LK, Olson NF (1982). Rheological Evaluation of Maturing Cheddar Cheese. J Food Sci. 47:631-638.

Creamer LK (1991). Electrophoresis of Cheese. Bulletion of the IDF, No:261, Chapter 4:14-26.

Farkye NY, Fox PF (1990). Objective indices of cheese ripening. Trends Food Sci. Tech. 1:37-42.

Feng Z, Zhang H, Zhang Y, Hu S, Jıang Q (2011). Optimization of Medium Composition for Production of Recombinant Calf Chymosin from *Kluyveromyces lactis* in Submerged Fermentation. J. Northeast Agric. Univ. (English Edition) 18 (1): 56-62.

Fox PF (1987). Cheese: An Overview. Cheese: Chemistry, Physics and Microbiology. (Ed: PF Fox) Vol. 1. Elsevier Applied Science, London, England.

Fox PF, Law J (1991). Enzymology of cheese ripening. Food Biotechnol. 5: 239-262.

Fox PF, Mcsweeney PLH (1999). Rennets: Their in Milk Coagulation and Cheese Ripening. Microbiology and Biochemistry of Cheese and Fermented Milk (Ed: BA Law). Blackie Academic and Professional, London, England.

Gripon JC, Desmadezaud MJ, Bars D, Bergere JL (1975). Etude des Role des Microorganismes et des Enzymes au Cours de la Maturation des Fromages. Le Lait, 55(548): 502-516.

Guinee TP, Wilkinson MG (1992). Rennet Coagulation and Coagulants in Cheese Manufacture. J Society Dairy Tech. 45: 94-104.

Irigoyen A, Izco JM, Ibanez FC, Torre P (2001). Infuence of rennet milk-clotting activity on the proteolytic and sensory characteristics of an ovine cheese. Food Chem. 72: 137-144.

Kosikowski FV, Mistry VV (1997). Cheese and Fermented Milk Foods. Vol. 1, Third Edition, Michigan, USA.

McSweeney PLH, Fox PF (1993). Contribution of indigenous microflora to the maturation of Cheddar cheese. Int. Dairy J. 3: 613-634.

Repelius K (1998). Coagulants produced by fermentation technology. Aust. J. Dairy Technol. 53: 124.

Rogelj I, Perko B, Francky A, Penca V, Pungercar J (2001).

Recombinant lamb chymosin as an alternative coagulating enzyme in cheese production. J. Dairy Sci. 84:1020-1026.
Salama FA, İsmail AA, Youssef AM, Salem SA (1982). Comparative studies on white pickled Brinza cheese made from cows' and buffaloes' milk in Egypt. Egyptian J. Dairy Sci. 10: 243-252.
Saldamlı I, Kaytanlı M (1998). Utilisation of Fromase, Maxiren and Rennilase as Alternative Cougulating Enzymes to Rennet in Turkish White Cheese Production. Milchwissenschaft 53: 22-25.
Silva S, Allmere T, Malcata FX, Andren A (2003). Comparative studies on the gelling properties of cardosins extracted from *Cynara cardunculus* and chymosin on cow's skim milk. Int. Dairy J. 13:559-564.
Şeker Ş, Beyenal H, Ayhan F, Tanyolaç A (1998). Production of microbial rennin from *Mucor miehei* in a continuously fed fermenter. Enzyme and Microbial Technol. 23:469-474.
TSE (1994). TS 1018 Standard of Raw Cow's Milk. Turkish Standard Institute, Ankara, Turkey.
TSE (1995). TS 591 Standard Ofwhite Pickled Cheese. Turkish Standard Institute, Ankara, Turkey.
Whyte NH (1995). Proteolysis of bovine and ovine caseins by lamb and calf chymosin. PhD Thesis, National University of Ireland, Cork, Ireland.