Journal of Food and Drug Analysis, Vol. 14, No. 3, 2006, Pages 279-283 藥物食品分析 第十四卷 第三期

Protein Properties of UF Teleme Produced from Various Types of Milk

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(Received: February 3, 2006; Accepted: May 8, 2006)

ABSTRACT

In this study, white cheese Teleme samples were produced from cow's, sheep's and goat's milk using UF and the traditional methods. In both methods, milk was divided into three portions and heated to 68°C/20 min, 72°C/5 min and 80°C/1 min., respectively. Each sample tested for three different pasteurization norms was divided into two portions. The calf rennet (CR) was added to the first portion and microbial rennet (MR) was added to the second portion. The teleme samples were prepared using 2 processing methods, 3 pasteurization norms,and 2 enzyme types. Besides total nitrogen and water-soluble nitrogen analyses, SDS-PAGE was applied to the samples to determine free protein breakdowns. Total nitrogen and water-soluble nitrogen values were higher in Teleme samples treated with microbial rennet than those with calf rennet in both UF and traditional Teleme samples. In the first day a rapid proteolysis and fraction product (casein fraction) just under the β-casein were determined in Teleme samples produced from milk with microbial rennet at 68°C and 72°C in SDS-PAGE. Data on water-soluble nitrogen fractions confirmed electrophoretic profiles.

Key words: Teleme, goat's milk, sheep's milk, ultrafiltration technique, SDS Page

INTRODUCTION

White pickled cheese is consumed freshly or processed straining, shaping and salting of curd formed after coagulating the milk by enzyme. This type of cheese is produced from sheep's, goat's and cow's milks. Like other cheese varieties, white cheese loses its major constituents during the whey drainage. In order to prevent this losts and increase cheese yield, UF technology is widely used in cheese making, Renner and Ömeroğlu[1]. Heat treatment of ultrafiltered milk also increases the cheese yield because it causes faster denaturation in retentate Renner and Abd El Salam[2], Espinoza and Calvo[3].

During cheese manufacturing, addition of enzyme to the milk for clotting is an important step. Chymosin is one of the most widely used enzyme for milk clotting. Nowadays, microbial rennets are produced by fermentation technique. It is known that proteolytic activity of microbial rennets is higher than that of chymosin as reported by Mc Mahon and Brown[4] and Ustunol[5].

In this research, the effect of UF treatment, different pasteurization temperatures and the use of curdling enzymes on the protein fractions of Teleme samples produced from cow's, sheep's and goat's milks were determined quantitatively and qualitatively. The protein values showed that Telemes produced from ultrafiltered cow's, sheep's and goat's milks subjected to different heat treatments and enzyme additions contribute to the improvement of cheese making techniques.

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MATERIALS AND METHODS

I. *Teleme Production*

This study was conducted at the Ankara University's Faculty of Agriculture Dairy Technology Department. Telemes were produced from cow, sheep and goat milks using the traditional method and ultrafiltration technique. In the traditional method, each milk type was divided into 3 equal portions and each portion was subjected to different heat treatments (68°C for 20 min, 72°C for 5 min and 80°C for 1 min). Then, each portion was further divided into 2 equal portions and either commercial liquid rennet consisting of 85% chymosin and 15% pepsin (Chr. Hansen's- Mandra Peynir Mayası) or microbial rennet (Chr. Hansen's-Yörük Peynir Mayası) produced from *Rhizomucor miehei* was added to portions. The curds were allowed to drain for 2-2,5 h.

In the UF method, fat-free milks were ultrafiltrated using a pilot GR61PP UF equipment (Dow Denmark A/S, Stavangervej 10 DK-4900 Nakskov, Denmark) with the polysulfone membrane area of 0.36 m^2. The capacity and the seperation degree were 10 L and 20 kDa, respectively. Fat-free milks were ultrafiltered under 8 bar inlet pressure and 1 bar outlet pressure at 35-45°C. The volume reduction (VR) of ultrafiltered cow, sheep and goat milks were approximately 70, 55, 70%, respectively. The Teleme samples from the ultrafiltered milk were produced using the same procedure used for the production of the traditional samples. The experiments were replicated.

II. *Determination of Protein Fractions*

Total nitrogen and water-soluble nitrogen contents of Teleme samples were determined quantitatively by Gripon *et al*[6]. The electrophoretic analysis was carried out by SDS-PAGE technique Laemmli[7]. The vertical type electrophoresis apparatus Bio-Rad Mini Protean III (Life Science Research Group 2000, CA 94547, Italy) was used in electrophoresis studies. All chemicals were obtained from Merck Chemical Company (Merck & Co., P. O. Box 100 Whitehouse Station, NJ 08889-0100 USA). The ripening index (RI) was calculated as the ratio of whey soluble (SN) to total protein (TN) using the formula,

$$RI = \frac{SN}{TN} \times 100$$

III. *Preparation of Teleme Samples for Electrophoresis*

LMV-SDS method proposed by Creamer[8] was modified to prepare samples. The prepared samples were diluted with sample buffer (1.5 g Tris, 2 g SDS, 2 mg bromophenol blue, 10 mL glycerol and 5 mL 2-β-mercaptoethanol /100 mL at pH 6.8) to give a final protein concentration of 1 mg/mL.

IV. *Preparation of SDS–PAGE Gel*

SDS–PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) gel was composed by separating and stacking gels at different pH and composition levels. In the preparation of separating gel, 2.5 mL of separating gel buffer (36.3 Tris/100 mL, pH 8.8), 4.7 mL of acrylamide–bisacrylamide stock solution, 3.18 mL of distilled water and 0.10 mL of 10% SDS solution were mixed. Then 5 µL of tetramethylene diamine (TEMED) and 50 µL of ammonium persulphate (APS) were added to the mixture.

For the preparation of stacking gel, 1.25 mL of stacking gel buffer (6 g Tris/100 mL, pH 6.8), 0.65 mL of acrylamide–bisacrylamide stock solution, 3.05 mL of distilled water and 0.05 mL of 10% SDS solution were mixed, then 5 µL TEMED and 25 µL of APS were added to the mixture. The mixture was then poured into the polymerised separating gel. Finally, a comb was installed into the separating gel in order to prepare the wells for inoculation of samples. When the polimerization was completed, the comb was removed and the wells were filled by the electrophoresis buffer solution (30 g of Tris, 144 g of glicyne and 10 g of SDS/ 5000 mL distilled water) and 20 µL of each Teleme sample was added on the gel in the wells.

The standard (Bio–Rad Catalogue No: 161-0304) was installed in the first and the last wells. Following the samples installation, direct current with 200 volts and 60 ampere from a power supply was applied to the system. After 40–45 min, gel was brought to the dying solution (1 g of Coomassie Blue 250 R, 400 mL of methanol, 100 mL of acetic acid and 1500 mL of water). The gel remained in the dying solution for 12 h. Then, it was kept in the destaining solution (400 mL of methanol, 100 mL of acetic acid/500 mL water) until its bands became apparent.

RESULTS

I. *Total Nitrogen (TN) and Water Soluble Nitrogen (WSN) contents of Teleme*

Table 1 shows the TN values of cow, sheep and goat Telemes. The UF processing increased the TN contents as expected. After the UF processing, Teleme produced by heat treatment at 80°C and by addition of microbial rennet have the highest nitrogen content in all samples (2.58%, 2.94% and 2.42% in cow, sheep and goat Telemes, respectively).

Both ultrafiltered and clotted rennet Telemes had the highest water-soluble nitrogen content in all samples. Microbial rennet had a rapid activity. It caused faster proteolysis than chymosin processed Telemes in traditional UF-processed Telemes, which were subjected to the heat treatment at 72°C.

II. *Electrophoretic Analysis*

SDS-PAGE electrophoregrams of cow, sheep and goat milk Teleme are shown in Figure 2. A fraction under the β-casein fraction was determined in the Teleme samples produced by traditional method by using microbial rennet and heating to 68°C for 20 min or at 72°C for 5 min. Similar situation was also observed in the SDS-PAGE of UF processed Telemes.

Although the α_s-casein and β-casein fractions are distinct in the cow and goat milk Telemes, the distinction of α_s-casein and β-casein is not clear in the sheep Teleme. Owing to high fat content of sheep milk, the fat was not separated completely in the sample preparation.

DISCUSSION

I. *Total Nitrogen and Water Soluble Nitrogen contents of Teleme*

Total nitrogen is a parameter used in the definition of protein content by Fox[9]. The Teleme samples heated to 80°C and clotted by microbial rennet after UF processing had the highest nitrogen content. McMahon *et al*.[10] reported that whey proteins remained in the coagulum due to the interaction of UF processing and heat treatment.

In fresh cheese, a part of casein was broken into water-soluble fractions such as proteose peptone, amino acids due to the effect of enzymes and starter cultures, Fox 1989[9]. High proteolytic activity of microbial rennet used in the manufacture of cheese causes the

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rapid fractionation in proteins. The milk Telemes with microbial rennet processed by UF showed higher whey protein nitrogen (WPN) values. This result may be explained as a reflection of protein degradation rate as a %-value in Telemes with have high protein content. Similar results were found by Hagrass *et al.*[11] and Ustunol[5] . Microbial rennet increased the WPN contents of Teleme samples at 72°C. On the other hand, WPN contents of the Teleme samples decreased at 80°C. Ustunol and Brown[12] explained that heat treatments at high temperatures (above 70°C) caused denaturation of whey proteins and some of the denaturated whey proteins formed a complex K-casein network. Thus, the activating ability of enzyme to this complex decreased.

II. *Evaluation of electrophoretic results*

In electrophoretic study, a band was observed in both

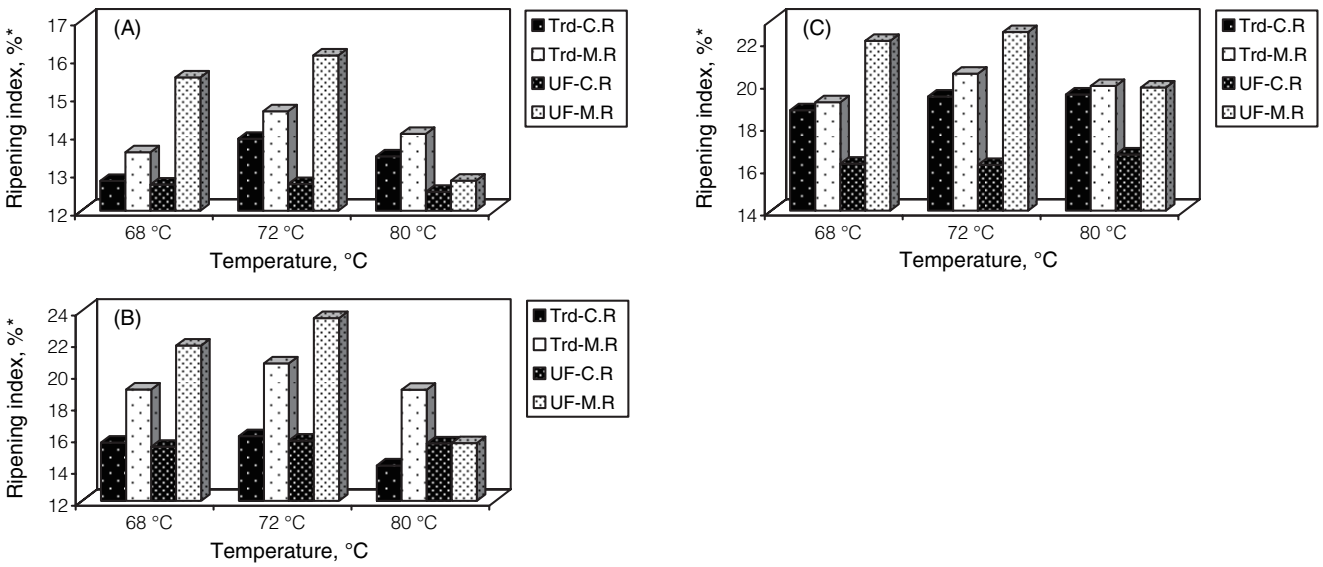

Figure 1. Ripening index of cow's (A), sheep's (B) and goat's Teleme (C).
Trd-C.R: Telemes produced by traditional way using calf rennet
Trd-M.R: Telemes produced by traditional way using microbial rennet
UF-C.R: Telemes produced by UF method using calf rennet
UF-M.R: Telemes produced by UF method using microbial rennet
* The ripening index was calculated as the ratio of whey soluble (SN) to total protein (TN)

Table 1. Total Nitrogen and Water-Soluble Nitrogen contents of cow's, sheep's and goat's Telemes (%) (n = 2).

Milk Type	Method*	Enzyme type	Heat Treatments					
			68 °C/20 min		72 °C/5 min		80 °C/1 min	
			TN**	SN***	TN**	SN***	TN**	SN***
Cow's	TRD	Rennet	2.19 ± 0.09	0.28 ± 0.00	2.16 ± 0.02	0.30 ± 0.03	2.16 ± 0.03	0.29 ± 0.05
	TRD	Microbial	2.29 ± 0.11	0.31 ± 0.07	2.19 ± 0.02	0.32 ± 0.05	2.14 ± 0.02	0.30 ± 0.05
	UF	Rennet	2.60 ± 0.19	0.33 ± 0.00	2.52 ± 0.25	0.32 ± 0.00	2.48± 0.14	0.31 ± 0.01
	UF	Microbial	2.58 ± 0.04	0.40 ± 0.00	2.55 ± 0.09	0.41 ± 0.02	2.58 ± 0.02	0.33 ± 0.03
Sheep's	TRD	Rennet	2.68 ± 0.12	0.42 ± 0.00	2.61 ± 0.06	0.42 ± 0.01	2.60 ± 0.21	0.37 ± 0.02
	TRD	Microbial	2.68 ± 0.02	0.51 ± 0.08	2.66 ± 0.09	0.55 ± 0.01	2.63 ± 0.16	0.50 ± 0.02
	UF	Rennet	2.92 ± 0.17	0.45 ± 0.02	2.91 ± 0.13	0.46 ± 0.03	2.88 ± 0.08	0.45 ± 0.01
	UF	Microbial	2.89 ± 0.22	0.63 ± 0.00	2.89 ± 0.23	0.68 ± 0.01	2.94 ± 0.31	0.46 ± 0.01
Goat's	TRD	Rennet	2.08 ± 0.14	0.39 ± 0.04	2.01 ± 0.19	0.39 ± 0.02	2.00 ± 0.18	0.39 ± 0.04
	TRD	Microbial	2.09 ± 0.03	0.40 ± 0.02	2.05 ± 0.02	0.42 ± 0.05	2.01 ± 0.08	0.40 ± 0.03
	UF	Rennet	2.34 ± 0.02	0.38 ± 0.01	2.35 ± 0.00	0.37 ± 0.01	2.28 ± 0.02	0.38 ± 0.01
	UF	Microbial	2.36 ± 0.09	0.52 ± 0.02	2.36 ± 0.18	0.53 ± 0.03	2.42 ± 0.27	0.48 ± 0.01

TRD: Traditional, UF: Ultrafiltration,
**Total nitrogen,
*** Water-soluble nitrogen

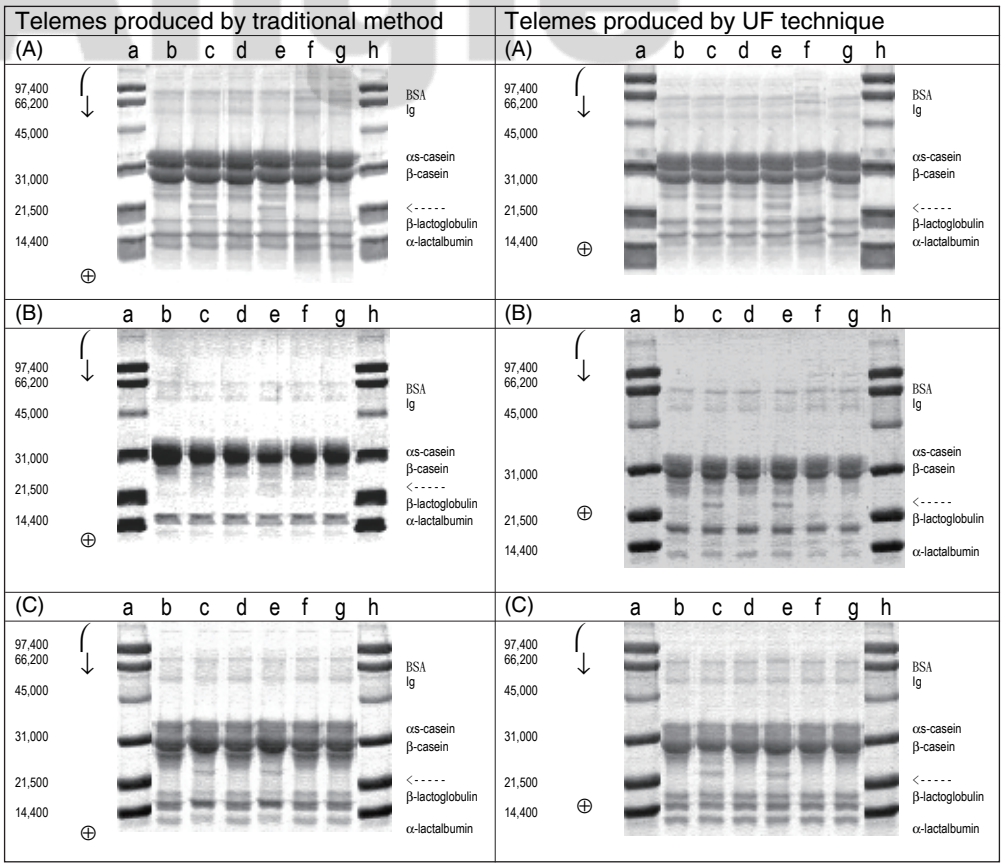

Figure 2. SDS-PAGE of cow, sheep and goat milk Telemes.
(A) cow's milk, (B) sheep's milk, (C) goat's milk (a) Standard, (b) 68°C/20 min-rennet, (c) 68°C/20 min-microbial rennet, (d) 72°C/5 min-rennet, (e) 72°C/5 min-microbial rennet, (f) 80 C/1 min.-rennet, (g) 80°C/1 min-microbial rennet, (h) Standard

the traditional and ultrafiltered Telemes with microbial rennet exposed to pasteurisation of 68°C and 72°C in the first day. This band formation in Telemes was an indication of a rapid proteolysis. This result was also confirmed by total nitrogen and whey protein nitrogen values. It was thought that this fraction was a degradation product of α_s-casein. Likewise, Buruiana and Farag[13] found that α_s-casein is degraded faster than β_s-casein in Teleme cheese. Similar results were also found by El-Shibiny *et al.*[14] and El-Safty and El-Shibiny[15]. Limiting effect of salt on the proteolysis is well known. However the Teleme samples were not put in brine solution for salting to facilitate proteolysis.

In this study, it was determined that ultrafiltration is a necessary procedure for the production of a good quality pickled cheese with high protein contents. The technique is not only used for cow's milk, but also for sheep's and goat's milks successfully. It is thought that heat treatment of milk at 68 - 72°C and the addition of microbial rennet in the proper amount gives a good result in the production of cheese. It was also determined that UF did not have an important affect on the proteolysis, whereas both enzyme type and heat treatment influenced the rate of proteolysis. Further studies containing ripening period will provide more practical results.

ACKNOWLEDGMENTS

This study was supported by the Ankara University Research Foundation (project no: 2000.07.11.023) and the Scientific and Technical Research Council of Turkey (TUBITAK, project no: TOGTAG/TARP-2398).

REFERENCES

1. RENNER, E. and OMEROGLU; S. 1981. Herstellung von Weiβkase aus ultrafiltrierter milch. Milchwissenschaft 36: 334-338.
2. RENNER, E. and ABD EL-SALAM, M.H. 1991. Application of ultrafiltration in the dairy industry. Chapter 9, Elsevier Applied Science, London and New York, 153-210.
3. ESPINOZA, N., A. and CALVO, M.M. 1998. Effect of heat treatment and ultrafiltration process cow's, ewe's or goat's milk on its coagulation properties. J. Agric. and Food Chem. 46: 1547-1551.
4. McMAHON, D. J. and BROWN, R.J. 1984. Enzymic coagulation of casein micelles a review. J. Dairy Sci. 67: 919-929.
5. USTUNOL, Z. 1993. Coagulation properties, proteo-

lytic activities of milk clotting enzymes, and their effect on cheese yield. Cheese Yield and Factors Affecting It's Control, IDF Seminar 267-273.

6. GRIPON, J.C., DESMAZAUD, M.J., LE BARS, D. and BERGERE, J.L. 1975. Etude du role des microorganismes et des enzymes au cours de la maturation des fromages. Le Lait. 55: 502-516.
7. LAEMMLI, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.
8. CREAMER, L.K. 1991. Electrophoresis of cheese. Bulletin of the IDF 261, Brussels-Belgium.
9. FOX, P.F. 1989. Proteolysis during cheese manufacture and ripening. J. Dairy Sci. 72: 1379-1395.
10. McMAHON, D. J., YOUSIF, B. H. and KALAB, M. 1993. Effect of whey protein denaturation on structure of casein micelles and their rennetability after ultra-high temperature processing of milk with or without ultrafiltration. Int. Dairy J. 3: 239-256.
11. HAGRASS, A.E.A., EL-GHANDOUR, M.A., HAMMAD, Y.A. and HOFI, A.A. 1983. Production of Ras cheese from recombined milk. III. Effect of some ripening agents. Egyptian J. Dairy Sci. 11: 271-279.
12. USTUNOL, Z. and BROWN, R.J. 1985. Effects of heat treatment and postreatment holding time on rennet clotting of milk. J. Dairy Sci. 68: 526-530.
13. BURUIANA, L.M. and FARAG, I. 1982. Changes of electrophoretic pattern of caseins in Telemea cheese during pickling in brine. 21th International Dairy Congress, Moscow.
14. EL-SHIBINY, S., MOHAMED, A.A., EL-DEEN, H.F., AYAD, E. and ABD EL-SALAM, M.H. 1979. Effect on protein breakdown in Ras cheese of the use of commercial enzyme preparations. Egyptian J. Dairy Science 7: 141-147.
15. EL-SAFTY, M.S. and EL-SHIBINY, S. 1981. The use of *Mucor pusillus* protease in the manufacture of Domiati cheese. Egyptian J. Dairy Sci. 8: 41 48.