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THE INFLUENCE OF ULTRAFILTRATION ON HYDROPHOBIC INTERACTIONS MILK PROTEINS IN THE PRESENCE OF FAT GLOBULES ON THE COURSE OF RENNETING

RENNETLENMEDE YAĞ GLOBÜLLERİNİN VARLIĞINDA SÜT PROTEİNLERİNİN HİDROFOBİK ETKİLEŞİMLERİ ÜZERİNDE ULTRAFİLTRASYONUN ETKİSİ

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ABSTRACT: In this study the skim milk and whole milk samples were concentrated for 2-fold by ultrafiltration (UF). Then the skim milk retentates were recombined with cream as such and 2-times protein / fat ratio of whole milk retentate. The modification of the milk proteins and fat globules was explained as the variation of protein surface hydrophobicity, and UF performance was explained as a result of collected permeat volume in giving time intervals during the ultrafiltration. It was also studied on renneting properties of these samples and their retentates. It was found that the number of hydrophobic sites of the whole milk's protein system lower than that of skim milk and it was observed at minimum in the whole milk's retentate. It is suggested that the renneting process accelerated due to the modification of protein structure resulted by UF and protein/ fat ratio.

Key words: Milk, proteins, fat, ultrafiltration, surface hydrophobicity, casein, renneting.

ÖZET: Bu çalışmada yağsız ve yağlı süt örnekleri ultrafiltrasyon (UF) ile 2 kat konsantre edilmiştir. Sonra yağsız süt retentatları krema ile yağlı süt retentatının protein/yağ oranı ile aynı ve 2 katı olacak şekilde rekombine edilmiştir. Süt proteinleri ve yağ globüllerindeki modifikasyon protein yüzey hidrofobisitesindeki değişim ile açıklanırken UF performansı ultrafiltrasyon boyunca belli sürelerde toplanan permeat hacmi ile açıklanmıştır. Ayrıca bu örneklerin ve retentatlarının rennetlenme özellikleri üzerine çalışılmıştır. Yağlı sütlerin protein sistemindeki hidrofobik kısımların sayısı, yağsız sütünkinden daha düşük bulunmuş ve yağlı süt retentatında minumuma ulaşmıştır. UF'den kaynaklanan protein yapısındaki modifikasyona ve protein/yağ oranına bağlı olarak rennetlenme sürecinin hızlandığı öne sürülmektedir.

Anahtar kelimeler: Süt, proteinler, yağ, ultrafiltrasyon, yüzey hidrofobisitesi, kazein, rennetleme.

INTRODUCTION

Application of the membrane processing technologies to the cheese industry is widely investigated (1, 2, 3). However, it is observed that there are some problems for semi-hard and hard cheeses manufactured from ultrafiltred milk (4, 5). Erdem (2000) (6) suggested that the size of micelles was decreased and they were possibly rearranged via hydrophobic bonds to a more compact micelle structure during the ultrafiltration process and it causes some physicochemical changes in the milk protein systems during the renneting. However, our knowledge on the changes of the milk proteins and fat of the whole milk during UF and its effect of manufacturing of cheese is limited. In whole milk, the fat is present in the form of globules which are surrounded and stabilized by their own membrane; it consists mainly of phospholipids and proteins (7). It is known that fat plays an important role in the characteristic cheese flavor, texture and acceptability (8). The concentration and the ratio of fat and casein are two very important parameters affecting cheese quality (9).

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This study aims to determine the influence of interactions between the milk proteins and fat globules on UF perfonmance and its effect on renneting.

MATERIALS AND METHODS

Raw milk samples were obtained from the institution farm, and they were skimmed in a lab scale centrifuge. Skim milk and whole milk samples were preserved by addition of sodium azide (2:10000), and stored at 4°C.

The ultrafiltration of the skim milk and whole milk samples was carried out by using an Amicon Model 8200 stirred-cell (Amicon GmbH, Germany) and Diaflo YM10 Membrane (nominal molecular weight cut-off the membrane was 10 kDa). All samples were concentrated 2-fold, as determined by the volume of permeate obtained. The cell was filled with 100 ml of the sample and then run until 50 ml of permeate was collected into a measuring flask. The volume of permeate obtained was recorded during the run. The skim milk retentates obtained after ultrafiltration were recombined with cream to the protein / fat ratio of the whole milk retentate and 2-times of it.

The protein content of the samples was determined according to Bradford Method (10).

The relative fluorescence intensity of the samples was measured by using a Perkin Elmer LS-50 spectrofluorimeter (USA) with a normal glass cell, at λ_{ex}=390nm, λ_{em}=480nm. 1,8-anilinonaphthalenesulfonic acid (ANS, Merck, Germany) was used as the fluorescent probe. Titration of protein solutions with increasing concentration of ANS provides information on both the hydrophobic number and affinity of binding sites. This type of application of fluorescent probe includes the determination of F_{max} and K_d of the "ANS-milk protein" complex (11).

The coagulation experiment was carried out according to Erdem (6).

RESULT AND DISCUSSION

The difference on protein surface hydrophobicity of the samples are given in Table 1. The protein and the fat contents of these samples are also given Table 2.

Table 1. The fluorescence parameters of the samples, (wm; whole milk, sm; skim milk, wm Ret; whole milk's retentate, sm Ret; skim milk's retentate, Rsm Ret; skim milk's retentate recombined with fat as such in whole milk's retentate, , 1/ 2 Rsm Ret; skim milk's retentate recombined with fat as 1/ 2 of whole milk's retentate).

	wm	sm	wm Ret	sm Ret	Rsm Ret	1/2Rsm Ret
F_{max}	116.8	261.7	33.7	119.7	79.8	94.5
K_d	14.7	12.5	19.8	14.3	19.9	18.1
F_{max}/K_d	7.9	20.9	1.7	8.4	3.9	5.2
[P]	24.7	24.8	59.8	51.3	51.3	51.3
PSH	0.32	0.84	0.03	0.16	0.08	0.10

Table 2. The protein (%) and fat contents (%) of the samples (wm; whole milk, sm; skim milk, wmret; whole milk's retentate, smret; sikim milk's retenate).

	Fat (%)	Protein (%)
wm	3.4	2.47
sm	< 0.1	2.48
wm ret	6.8	5.98
sm ret	0.1	5.13
cream	60	*

The number of the surface hydrophobic sites (F_{max}) and the binding affinity of ANS to proteins ($1/K_d$) decreased with presence of fat globules in unconcentrated whole milk samples when compared with the unconcetrated skim milk samples. Also, both of the average tightness of binding of ANS to the protein (F_{max} / K_d) and the protein surface hydrophobicity index (PSH) decreased. It is explained that in the number of collision between the micelles increases with decreasing protein / fat ratio of the milk.

It is observed that the drastic changes were occured in the presence of fat globules in the concentrated milk samples (Figure 1 and Table 1). The difference in PSH between the concentrated whole milk and other samples can be explained as the fat globules playing a role as a masking agent on the modified surface of the proteins (Figure 2).

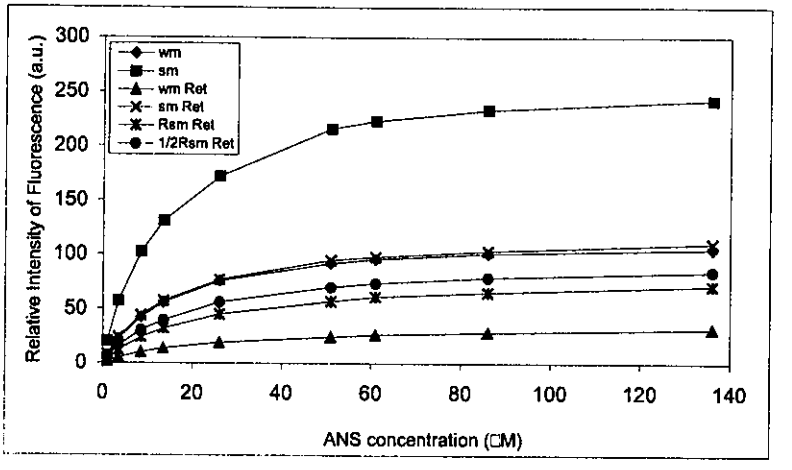

Figure 1. **ANS titration curves of the samples (wm; whole milk, sm; skimmilk, wmRet; whole milk's retentate, smRet; skimmilk's retentate, RsmRet; skim milk's retentate recombined with fat as such in whole milk's retentate, 1/2RsmRet; skim milk's retentate recombined with fat as 1/ 2 of whole milk's retentate)**

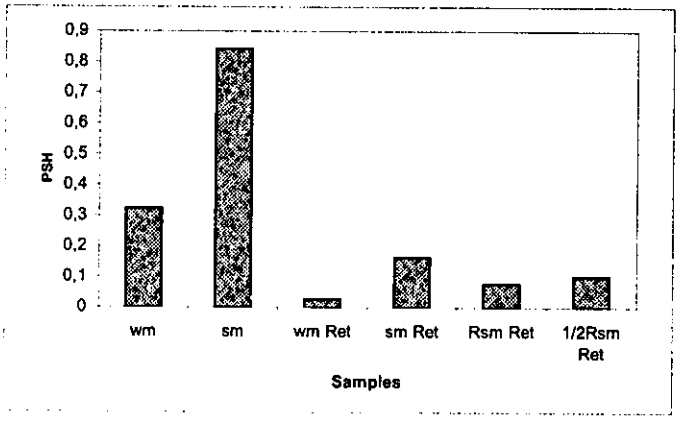

Figure 2. **Protein surface hydrophobicity indexes of the samples (wm; whole milk, sm; skimmilk, wmRet; whole milk's 2-fold UF retentate, smRet; skimmilk's 2-fold UF retentate, RsmRet; skimmilk retentate recombined with fat as such in whole milk's retentate, 1/2RsmRet; skim milk's retentate recombined with fat as 1/ 2 of whole milk's retentate)**

It was observed that the changes which were occurred at the number of surface hydrophobic sites (F_{max}) was lower in the whole milk retentates than that of the skim milk retentates. The changes of protein surface hydrophobicity by UF increased with decreasing protein / fat ratio of the milk samples. However, the small changes in F_{max} and K_d which were obtained in concentrated skim milk and unconcentrated whole milk samples were not significant ($p<0.01$).

It is observed that the recombined retentates showed apparent differences on the surface hydrophobicity when compared to the skim milk retentates. However, F_{max} and PSH value increased while increasing protein / fat ratio (1/2 Rsm Ret). The difference in PSH value between the skim milk retentate and 1 /2 Rsm Ret samples was less.

The highest masking effect of protein surface hydrophobic patches occured in whole milk retentates.

Consequently, it suggests that there are some rearrangements between the milk proteins and fat globules during the UF. The milk protein system was reorganized to a more compact structure by UF, and this modified structure of the proteins gets stronger when the fat globules are present.

The UF performance of the samples are also appropriate with the results above mentioned. As is shown in Figure 3 (UF performance), the whole milk samples showed a higher permeat flow rate than that of the skim milk samples. It means that compact complexes of bigger sizes between milk proteins and fat globules occurred during the UF.

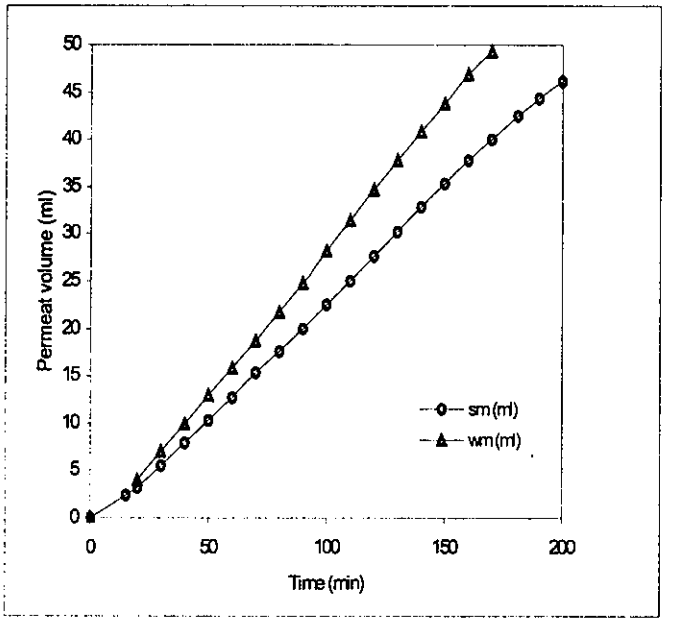

Figure 3. Permeat flow rate of the skim milk (sm) and whole milk (wm) samples

Table 3. Coagulation parameters of the samples (t0.8, in minutes) (wm; whole milk,. sm; skim milk, wm2xret; whole milk's retenate, sm2xret; skim milk's retenate, sm2xretRec; skim milk's retentate recombined with fat as such in whole milk's retentate, sm2xret1/2Rec; skim milk's retentate recombined with fat as 1/2 of whole milk's retentate).

	$t_{0.8}$ ANS Partiton		$t_{0.8}$ Protein Partiton	
Samples	ppt	sn	ppt	sn
wm	33.6	26.2	43.6	15.2
sm	38.4	32.4	37.8	33.5
wm 2x-ret	15.6	7.20	7.60	19.4
sm 2x-ret	40.4	36.0	34.9	38.0
sm 2x-ret Rec	38.0	11.6	7.80	12.2
sm 2x-ret 1/2 Rec	23.1	14.5	18.2	19.6

It was suggested that large macroparticules are retained on the membrane surface and protect the membrane pores from fouling during the UF (12). The presence of fat globules affects the permeat flow rate during the UF. The milk proteins in the presence of fat globules play a 'sievig role' on the membrane surface which accelerates the permation.

Changes of coagulation pattern of the samples explained. The results of ANS partition and protein partition of the samples during the renneting are shown in Figures. 4 and 7. The times required to reach on 80% completion of the process were determined ($t_{0.8}$, minute) for partition of ANS and protein according to the method of Erdem (2000) (6) and are given in Table 3, in minutes.

When 80% of the total k-casein has been hydrolysed, the micelles began to aggregate progressively into a gel network in raw milk (13).

It is shown from Table 3 that the time required for binding ANS when 80% of the total k-casein has been hydrolysed ($t_{0.8ANS\ Partition}$) decreased significantly in whole milk's retentate altought it slightly increased in skim milk's retentate. However, $t_{0.8ANS\ Partition}$ increased in recombined skim milk retentates and it decreased with increasing protein / fat ratio.

$t_{0.8ANS\ Partition}$ Partition is an important index because it informs the level or rate of hydrolization of the casein into para-casein during the renneting. It is known that ANS binds spreaded of hydrophobic patches with enzymatic clotting.

There are also some differences in protein partition ($t_{0.8ANS\ Partition}$) between the whole milk retentate and skim milk retentate (Table 3). $t_{0.8ANS\ Partition}$ decreased severely in the whole milk's retentate and recombined retentates. It means that the time required for 80% of the total k-casein has been hydrolysed decreased and the rate of hydrolization increased in the whole milk retentate. The caseins and the fat globules may be interact during the UF. This structure occurred during the UF is probably caused a decreasing on the required enzyme quantity and the renneting time.

It is reported that the renneting process gets faster with increasing of protein concentration in the skim milk by UF (6). As is shown in Table 3 and Figure 4 - 7 the fastest renneting process occurred in retentates when the

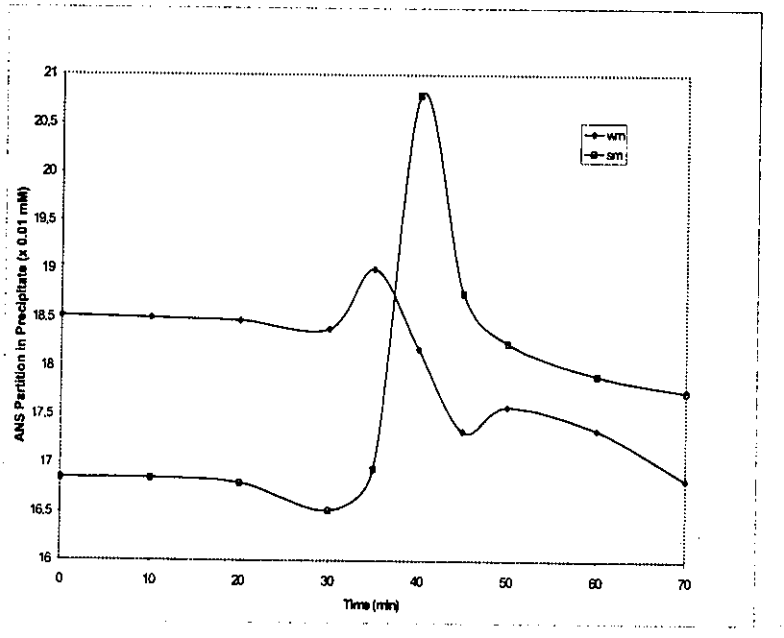

Figure 4. ANS partition per protein in precipitates of unconcentrated milk samples (wm; whole milk, sm; skimmilk).

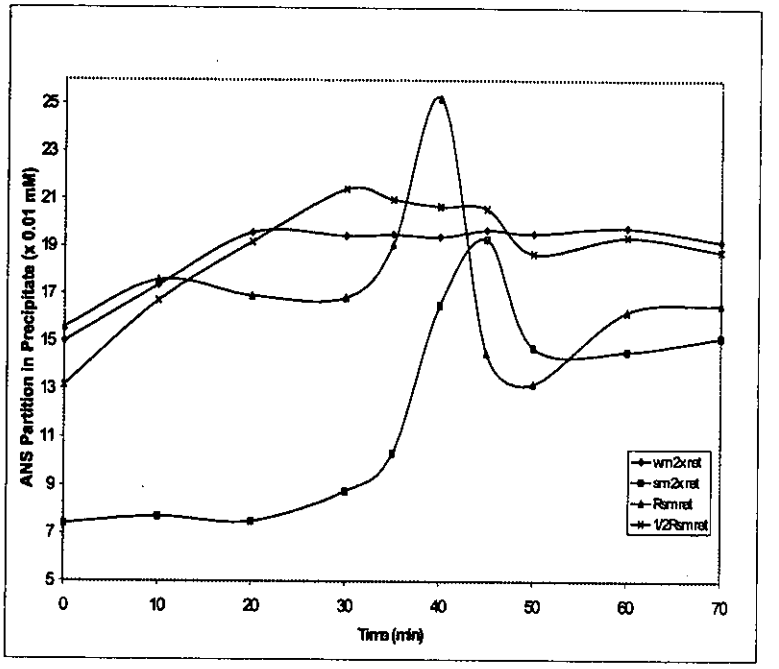

Figure 5. **ANS partition per protein in precipitates of the retentates (whole milk's retentate; wm2xret, skim milk's retentate; sm2xret, Rsmret; skim milk's retentate recombined with fat as such in whole milk's retentate, 1/ 2 Rsmret; skim milk's retentate recombined with fat as 1/ 2 of whole milk's retentate).**

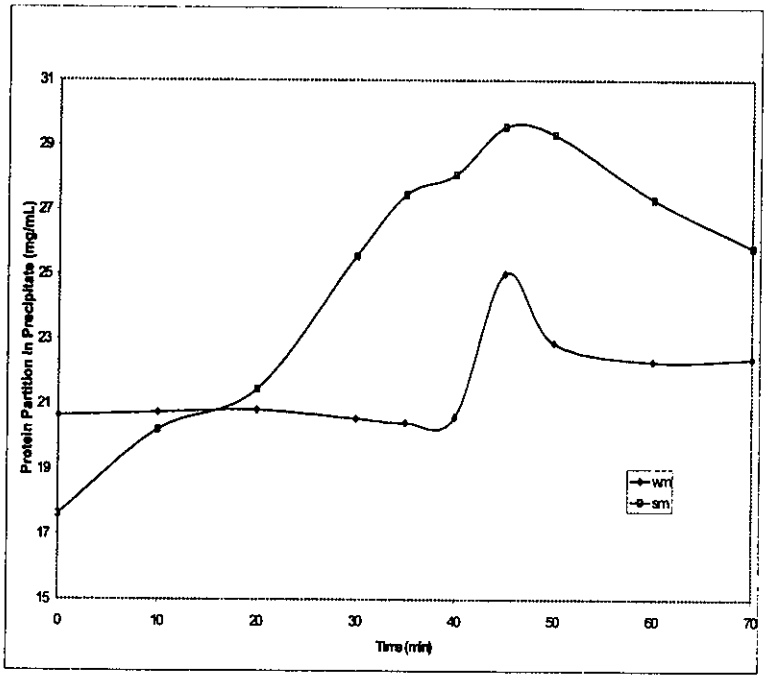

Figure 6. Protein partition in precipitates of the unconcentrated milk samples (wm; whole milk, sm; skim milk).

fat globules were present. The time required for protein partition ($t_{0.8Protein\ Partition}$) decreased to 34.9 min than 37.8 min in skim milk while decreasing to 7.6 min than 43.6 min in whole milk which were concentrated by UF. It was decided that the changes which were occured during the ultrafiltration of milk would be useful to manufacture of cheese from whole milk retentate and recombined skim milk retentate.

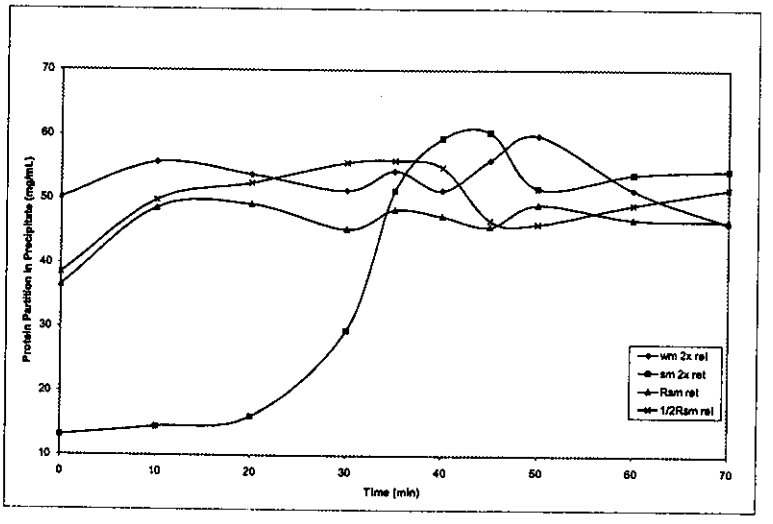

Figure 7. **Protein partition in precipitates of the retentates (whole milk's retentate; wm2xret, skim milk's retentate; sm2xret, Rsmret; skim milk's retentate recombined with fat as such in whole milk's retentate, 1/ 2 Rsmret; skim milk's retentate recombined with fat as 1/ 2 of whole milk's retentate).**

CONCLUSIONS

The objective of this study was to explain the effect of UF on the milk protein system in the presece of the fat globules. When the fat is removed (skimmed) from the milk, the number of surface hydrophobic sites is increased apparently (see Table 1). The affinity and tightness of hydrophobic interactions are also increased with skimming.

The influence of ultrafiltration on hydrophobicity of milk filtration system (F_{max}; $1/K_d$;, F_{max} / K_d ;, PSH) was as decreasing. In both cases above mentioned, the micelle structure is modified to a more closed patches. Fat possibly plays a masking role on the micelle surface which causes a poor possibilty of collision between the micelles, and UF results more compact micelle structure. If the fat which was removed from the milk before is recombined to the retentate in ratio which is as in whole milk's retentate, the resultant hydrophobicity is not reached back to the original level. The PSH index (overall hydrophobicity) of the recombined skim milk's retentate was found higher than that of whole milk's retentate. It means that the presence of fat causes a surrounded and bigger micelle structure, and that micelles cause the acceleration of permeation during the ultrafiltration.

On the other hand the renneting process is also affected by fat. The coagulation pattern of the skim milk is reached to a maxima faster than whole milk. When skimm milk's retentate is recombined with cream as protein/ fat ratio of whole milk's retentate, the coagulation is being faster than the other samples, and it decreases with increasin fat content gradually. The ultrafiltration process affects the renneting in whole milk more than skim milk. The number of open hydrophobic sites which were located on para-casein (hydrolized by rennet) micelles decreased with the presence of fat and with the concentration by ultrafiltration. But UF has a compacting effect on the micelles which causes a decrease in the length of collision and in the volume between the micelles. As a result of this, it accelerates the renneting, but fat has got a retardationon it.

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APPENDIX

F_{max} , the number of surface hydrophobic sites

F_{max} / K_d ; the average tightness of binding of ANS to the protein

$1/K_d$; The binding affinity of ANS to the proteins

K_d ; the ANS concentration in the case of $F_{max}/2$ due to Michealis-Menten kinetics and it denotes the dissociation constant of the fluorescent ANS-protein complex

PSH ; protein surface hydrophobicity index

$PSH = F_{max}/K_d$.[Protein Concn.]

REFERENCES

1. Green ML, Turvey A, Hobbs DG. 1981. Development of structure and texture in Cheddar cheese. J Dairy Res, 48: 343-355.
2. Leievre J, Lawrence RC. 1988. Manufacture of cheese from milk concentrated by ultrafiltration. J Dairy Res, 55: 465-477.
3. Rao DV, Renner E. 1988. Studies on the application of ultrafiltrationfor the manufacture of Cheddar cheese. 2. Effect of heating UF whole milk concentrate on composition and yield. Milchwiss, 43: 708-711.
4. Fox PF. 1987. Cheese manufacture: chemical, biochemical and physical aspects. Dairy Industry Int, 52: 11-130.
5. Grandison, AS, Glover FA. 1994. Membrane processes of milk. In *Modern Dairy Technology*, RK Robinson (ed), vol 1, UK: Chapman & Hall, 485 p.
6. Erdem, YK. 2000. Influence of ultrafiltration on modification of surface hydrophobic sites of the milk protein system in the course of renneting. J Food Eng, 44: 63-70.
7. Dalgleish, DG, Banks JM. 1991. The formation of complexes between serum proteins and fat globules during heating of whole milk. Milchwiss, 46: 2, 75-78.
8. Rodriguez, J, Requeana, T, Fontecha J, Goudedranche H, Juarez, M. 1999. Effect of different membrane seperation technologies (ultrafiltration and microfiltration) on the texture ad microstructure of semihard low-fat cheeses. J Agric and Food Chem, 47: 558-565.
9. Fox, PF, Mc Sweeney PLH. 1998. *Dairy Chemistry and Biochemistry*, Chapman and Hall, London, 478p.
10. Bradford, MM. 1976. A rapid and sensitive method for quantitation of microgramquantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72: 248-254.
11. Bonomi, F, Iaemetti S, Pagliarini, E, Peri, C. 1988. A spectrofluorimetric approach to the estimation of the surface hydrophobicity modifications in milk proteins upon thermal treatment. Milchwiss, 43: 281-285.
12. Erdem, YK, Yuksel, Z. 2001. Influece of heat treatment of milk before ultrafiltration on the membrane performance, Proc. on 4th WISA-MTD Symposium Membrane; Science & Engineering, Stellenbosch, RSA, p.58.
13. Walstra, P, Jennes, R. 1984. *Dairy Chemistry and Physics*, John Willey and Sons Inc, NY, 407 p.