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# Efficiency and Ropiness of Traditional White and Ultrafilteration Cheese as Affected by *Psycrotroph* Bacteria

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During cheese ripening, some of water insoluble casein change to water soluble nitrogen which include intermediate hydrolyzate protein and free amino acids. In this work, ripening and efficiency of traditional white cheese and ultrafilteration cheese as affected by *Psycrotroph* bacteria was investigated. The result showed that amount of *Psycrotroph* bacteria on the white and ultrafilteration cheese was significant. Also, there was a good correlation between *Psycrotroph* bacteria and ratio of soluble nitrogen to total nitrogen during ripening period. In fact, as the *Psycrotroph* bacteria increase, the ratio of soluble nitrogen to total nitrogen increased during ripening period. The per cent of N recovery (efficiency index) for ultrafilteration and traditional white cheese was 88.01-90.45 and 77.23-80.52, respectively. The effect of changing above parameter on ultrafilteration cheese is more than white cheese.

**Key Words: Ripeness, Traditional white cheese, Ultrafilteration, *Psycrotroph* bacteria.**

## INTRODUCTION

Cheese protein was hydrolyzed by rennet enzyme, starter bacteria and natural milk proteases which produce high and low molecular weight of peptides, amino acid, amine, aldehyde and sulfur compound. With regarding to complication of proteolyse and main goal may be used specified and non specified method for its evaluation. Non-specified method is somewhat simple and for routine measurement of cheese ripening is valuable. It should be noted that soluble nitrogen has high relation with cheese age and less with cheese quality. During cheese ripening non-protein nitrogen will be significantly increased due to proteolysis. So non-protein nitrogen is 2.1 % in the first day, but after three months, it will increase to 28.6 %. The changes in amounts of free amino acids also occur in the ripening process. Proteolysis play the most important role in producing aroma and taste in cheese which is comes from peptides and free amino acids and aroma compound such as amine, acid triol and trioester[1-3].

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One of the most important changes in dry matter is protein dehydration. As the polar group is high in the protein latex, dehydration will be high and as a result the dry matter reduces. It should be noted that proteolysis produce polar group such as amino acid, peptides, amine and carboxyl group that makes the solubility and hydration of protein increased. As the proteolysis is stronger, the dry matter of cheese reduced due to water absorption. The effective factors in cheese ripening are include: (i) **Time:** cheese ripening in the initial steps is more than final ripening (more than of 2/3 of ripening spend in the 3 first mounts). (ii) **Temperature:** as the temperature increased the ripening is more. (iii) **Moisture:** the more moisture, the more ripening. (iv) **Size of cheese mold:** ripening will be increased as the size of mold increases. (v) **Salt:** by increasing the salt, ripening reduces. (vi) **The amount of rennet:** as the rennet increased, the ripening will be more, but undesirable effect is also more. (vii) **pH:** ripening accelerate with low pH.

Generally, for evaluation of cheese ripening, the intense of hydrolysis of protein is measured. Proteolytic enzymes come from *Psycotroph* bacteria in milk made so many problems arises in dairy products such as undesirable aroma, weakening the structure of curd during the cheese making and reduction of efficiency. These enzymes also cause the UHT milk gelatinized. It is also reported that growth of *Psycrotroph* bacteria in milk cause the reduction of coagulation time. Many researches carried out on cheese efficiency. In all these investigations, reduction of cheese efficiency with increase of *Psycrotroph* bacteria was confirmed. It is also stated that the efficiency of cheedar cheese is reduced by increasing number of *Psycrotroph* bacteria in raw milk, which is related to decomposition of protein and fat[2-4].

## EXPERIMENTAL

All the chemicals used in these experiments were Merck. The place of experiment was conducted at Pegah milk factory, eastern Azerbaijan. Incoming raw milk after some quality experiments (acidity, fat, density, pH, moisture content and temperature) was accepted.

**Milk sample:** Raw milk was prepared from winter season. The milk used in the cheese making factory in the Pegah of east Azerbaijan of Iran. Rennet used in production of ultrafilteration cheese and white cheese was from of fromase. The starter was mix of termophile starter type yoghurt 709, yoghurt 231 and mesophile starter type $G_3$ mix 6, $G_3$ mix 7 that prepared in the ratio of 7 to 1, respectively.

**Procedures:** Total plate count (TPC) of raw milk was determined according to Iran standard number 356. Amount of *Psycrotroph* bacteria was measured according to Iran standard number 3451. The regression of coefficient ($R^2$) for cheese properties was determined by SPSS, Excel and Quattropro software. Total nitrogen of cheese was measured by Kjeldal method by using of block digestor/steam distillation. The amount of sample for measuring protein cheese was 2 g and total proteins were calculated by multiplying total nitrogen to 6.38. 40 mL of 0.5 molar sodium citrate that pH keep constant to 7 by use of 1 N HCl, was added to 10 g of cheese and heated in the water bath 40 ºC.

Cheese was crushed by crasher during 2 steps of 30 s and the temperature reached to 224 ºC. After that the solution aliquot reached to 200 mL. Add normal hydrochloric acid drops by drops to the 150 mL of above citrate solution till the pH solution reached to 4.4. Mix the solution for 20 min in room temperature and if it needs justify the pH and then the solution reached to 200 mL. This solution clarified by Whattman No. 42. The filtrate has soluble nitrate and measured by Kjeldal method. For estimation of efficiency of ultrafilteration cheese and white cheese used the following equations. Therefore the % of nitrogen of milk and cheese calculated in a day[5].

## RESULTS AND DISCUSSION

The effect of number of *Psycrotroph* bacteria on ripening factor of cheese and the amount of soluble nitrogen to total nitrogen was surveyed. Data related to ripening factor for normalization was evaluated by primary method[5]. The results showed that between the *Psycrotroph* bacteria and increase of soluble nitrogen to total nitrogen during ripening period was a significant relation. It means when the *Psycrotroph* bacteria increase, the ratio of soluble N to total N will be increased. The ratio of soluble N/total N by increasing the *Psycrotroph* bacteria was increased after 2 weeks in the traditional white cheese[6].

In order to compare the increasing per cent of the ratio of soluble N/total N during ripening, the average of this factor was compared by t-student in the 3 periods of time in the white traditional cheese.

The results showed that the average of soluble N/total N at 4th week was more than 2nd week and 6th week was more than 4th week. It means total soluble nitrogen depends on time of ripening. In fact, *Psycrotroph* bacteria in cheese induce enzymes which make proteolysis of protein and therefore the soluble nitrogen was increased. In order to know, how many *Psycrotroph* bacteria have a significant effect on the soluble nitrogen, the amount of bacteria was evaluated. If the plate count of *Psycrotroph* bacteria in raw milk was lower than 8000000 in 1 mL, effect of *Psycrotroph* bacteria on soluble nitrogen to total nitrogen was not significant in the white traditional cheese. While plate count of more than 8000000 in raw milk, per cent of soluble N to total N was significant within the 2nd and 4th week cheese ripening. So, when the total of *Psycrotroph* bacteria increased to 8 millions or more, sensible grow was occurred on soluble N to total N.

After 6 months of ripening period, the soluble N compare to 1 month cheese showed a significant increase. Data showed normal distribution. Regression of effect plate count of bacteria on ripening factor of ultrafilteration cheese was investigated. There was a significant correlation between *Psycrotroph* bacteria and soluble N to total N in the 2nd week, 4th week and 6th week of ultrafilteration cheese ripening.

It means, by increasing bacteria count, the soluble N to total N increased and $R^2$ increased. It may be seen that by increasing *Psycrotroph* bacteria in raw milk and passing time, more protein hydrolysis and soluble nitrogen was increased. This

process was occurred as a result of proteolysis by proteolytic enzymes produced by *Psycrotroph* bacteria. The soluble nitrogen was maximum in the 6th week of ripening. For determining that how many *Psycrotroph* bacteria have a significant effect on increasing soluble nitrogen, total count of bacteria was measured in different weeks.

By comparison per cent of soluble N to total N in 4th week and 2nd week cheeses, it may found that the total count of *Psycrotroph* bacteria in raw milk was lower than 7 million per mL. There was no significant difference between 1st month and 2nd week. On the other hand, with increasing *Psycrotroph* bacteria to 7 million CPU/mL or more, the soluble nitrogen will be increased. There was a significant difference between soluble nitrogen of cheese in the 6th week and 4th week, which prepared from raw milk have 7 million cfu/mL *Psycrotroph* bacteria or more. This process may be related to proteolytic activity of their proteolysis enzymes. Many researches showed that soluble nitrogen at pH = 4.6 was including medium and little peptides and amino acids[4-7].

Other studies carried out on cheddar cheese showed that if the *Psycrotroph* bacteria was more in raw milk, soluble nitrogen in solution with pH = 4.6 and nitrogen in TCA were 568-848, 230-465 mL in 100 g cheese, respectively. According to the investigation, proteasis of *Psycrotroph* bacteria cause to release plasmin and plasmiogen from micelle of casein and the soluble N increases and therefore affected the quality of dairy products. It is also studied on decomposing of casein by microbial proteasis in the 4 ºC milk stored in silo and showed that the proteolytic activity of *Psycrotroph* bacteria on β-casein had a significant correlation with increasing the time of stored milk. Therefore, after 4 d of storage milk at 4 ºC, the amount of β-casein was lowered to 50 %.

Another investigation showed that a significant relationship between *Psycrotroph* bacteria and multiplying soluble N and reduction of cheese efficiency as basis of dry matter[6,7].

In the ultrafilteration cheese processing, these effects through the membrane separating properties increase the per cent of recovery nitrogen or total proteins. Some investigators reported that soluble nitrogen of cheddar cheese increased by multiplying *Psycrotroph* bacteria in the raw milk and also stated that proteolytic enzymes of *Psycrotroph* bacteria will be survived after heat treatment and cause to proteolysis of protein and soluble nitrogen which are including small peptides and amino acids are produced[8,9].

The ratio of soluble N to total N of cheese was measured after 45 d, 2 weeks and 1 month and compared by t-student. there was a significant difference between multiplying soluble N after 2, 4 and 6 weeks. The soluble N in ultrafilteration cheese was more than traditional white cheese during ripening. There was a significant difference among the 2 type of cheese in three periods of ripening. The coefficient of regression was calculated for both of ultrafilteration and white cheese. The soluble N increased as *Psycrotroph* bacteria increased. The increasing of soluble N in

ultrafilteration cheese was more than white cheese. As an instance, the slope of curve was 2.094 and 3.26 % in the 6 week for traditional white cheese and ultrafilteration cheese, respectively. It means that by multiplying 1 million *Psycrotroph* bacteria, the soluble N to total N was increased 2.094 and 3.26 % in white and ultrafilteration cheese, respectively.

The firmness of texure of white cheese and ultrafilteration cheese was evaluated by pressure and cutting experiments. Total plate count (TPC) and plate count of *Psycrotroph* bacteria was 17 and 13 millions in raw milk, respectively. On the other hand, the 70 to 80 % TPC contains *Psycrotroph* bacteria and therefore these bacteria was dominant in raw milk. First the normalization of data was conducted by some researchers and the per cent of nitrogen recovery (efficiency coefficient) was calculated 88.01-90.45 and 77.23-80.52 in ultrafilteration cheese and white cheese, respectively[10]. The equation had -0.043 coefficients in white traditional cheese. It means that by multiplying 1 million *Psycrotroph* bacteria, nitrogen recovery reduced 0.043 in white cheese, but for ultrafilteration cheeses this coefficient was -0.028.

The per cent of nitrogen recovery in ultrafilteration cheese was more significant than traditional cheese. Some experts reported that effect of TPC on recovery of nitrogen did not exist for ultrafilteration processing. Whereas other showed that the population of *Psycrotroph* bacteria specitially *Pseudomanas* was so much. The effect of these enzymes on quality and efficiency of cheese was more significant when the TPC reached to 106 cfu/mL[8]. The studies showed a significant correlation between *Psycrotroph* bacteria and soluble N and reduction of efficiency of traditional cheese according to dry matter basis. These effects with regarding to membrane separation status in the ultrafilteration cheese processing through the achievement of nitrogen recovery or total nitrogen was induced. According to the investigations, proteasis of *Psycrotroph* bacteria cause to release plasmin and plasmiogen from micelle of casein and the soluble N increases and therefore affected the quality of dairy products. There was a significant relation between holding time of raw milk and *Psycrotroph* bacteria and reduction of cheese efficiency. The average of efficiency reduction reported that 0.5 % or every day of holding at 5º may be related to proteolysis and lypolysis process[8,9].

## Conclusion

The overall results showed that growth of *Pseudomanas* and *Flavobacterium* in raw milk make to reduce the efficiency to 1.4-4.2 % and reduction of N efficiency was 0.53 and 0.39 % for every day holding for *Pseudomanas* and *Flavobacterium*, respectively and the most reduction occurred when the *Psycrotroph* bacteria population reached to $10^6$-$10^7$ cfu/mL[8-10].

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