

Studies on the Effect of Starter Culture Concentration and Renneting pH on the Iranian Brine Cheese Yield

¹Masoud Najaf Najafi, ²Arash Koocheki and ¹M. Mahdizadeh

¹Department of Food Science and Technology,

Jihad Agriculture Hasheminejad Higher Education Center, Mashhad, Iran

²Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract: The cheese yield has a major importance on quality of final product. Among the different factors, the role of starter culture and renneting pH is of an importance in making of Iranian brine cheese. Thus, the effects of commercially available starter culture which is a mixture of mesophilic (*Streptococcus lactis*, *lactis* and *Str. lactis. cremoris*) and thermophilic (*Str. thermophilus* and *Lactobacillus bulgaricus*) bacteria and renneting pH on the physico-chemical changes of Iranian brine cheese was investigated throughout ripening and storage up to 5 weeks. The experiment works have been done into 3 levels for starter culture amount (1, 2 and 3% w/w milk) and pH of renneting (6, 6.2 and 6.4). The rate of pH decrease was the fastest in cheeses made with 3% starter culture and renneting pH of 6 and the slowest in cheeses made with the 1% culture and renneting pH of 6.4. The acidity was higher in cheese made with 3% starter culture. The highest fat content was in cheeses made with 3% starter culture and renneting pH of 6.4 and the highest yield in fresh cheese was in cheese made with 2% culture and renneting pH of 6. Starter culture concentration and renneting pH had significant impact on salt content in cheese where as total solid was not affected by starter culture concentration. During ripening, culture concentration and pH of renneting significantly affected salt content in cheese.

Key words: Cheese · rennet · starter · yield

INTRODUCTION

Cheese is one of the most versatile foods suitable for all age groups which can be consumed in many different meal occasions [1]. Traditionally, cheese has been regarded by the consumers as a nutritious food because it is a source of high quality proteins, dietary calcium, fat and other nutrients. The basic composition and structure of cheeses are determined by the manufacturing operations like pH at renneting, but it is during ripening that the individual and unique characteristics of each cheese variety develop [2].

Iranian brine cheese is probably the most popular and economically important traditional cheese in Iran. As this cheese is usually produced by traditional methods, problems such as non-uniform quality and flavour and texture defects are often encountered. Iranian White cheese is a close textured brined cheese [3] made from cow's milk, sheep's milk or mixtures of them. The

main flavor characteristics are acidity and saltiness [4]. This cheese is widely consumed all over Iran as a major diet in breakfast and manufacturing of other domestic cheese varieties such as jug cheese [3] and also processed cheese.

Traditionally, brine cheese is manufactured from non-pasteurized milk in small family premises with basic equipment. However, the use of raw milk leads to unpredictable chemical or biological changes or possible survival of various pathogens during manufacture and ripening [5]. Nowadays, pasteurized milk is used instead of raw milk in organized cheese dairies making necessary the use of Lactic Acid Bacteria (LAB). The widespread use of commercial starter cultures in cheeses manufactured from pasteurized milk has resulted in the loss of typical characteristics peculiar to different cheese types.

In Feta and Iranian cheese production, sometimes traditional yogurt is used as starter by the traditional cheese-makers. However, compared with the same

cheeses made from raw milk, replacement of the complex microbial flora present in raw milk by standard starter culture strains tends to result in uniform product characteristics [6]. Thus, it is preferable that a starter culture is used in cheese production.

The fact that white brined cheeses are traditionally produced with no starter cultures at all is one of the causes of its frequent indifferent quality. The selection, maintenance and use of starter culture are perhaps the most important aspect of cheese making, particularly in the context of modern mechanized processes where predictability and consistency are essential. The use of starter culture leads to the inhibition of growth of undesirable bacteria, improved whey drainage, production of characteristic flavours and aromas and controlled acid development and also aids the action of rennet by reducing pH [7]. However, in most cases, commercial cultures are not suitable for the production of traditional cheeses, as insufficient flavour and texture defects can be observed in cheeses prepared using such starters [8]. The chemical components of a cheese are derived from milk components and non-cheese milk ingredients such as cultures and enzymes.

Various adjunct cultures have been used, e.g. adjuncts isolated from other cheeses [9-11], or commercially purchased adjunct cultures [12].

The objectives of the present paper were to 1) study the effects of different concentration of commercially available starter culture (mixture of mesophilic and thermophilic bacteria) and different renneting pH on the physico-chemical changes of Iranian brine cheese and 2) to increase the yield of Iranian brine cheese.

MATERIALS AND METHODS

Cheese-making: Iranian brine cheese was manufactured using fresh raw milk which was obtained from a dairy farm in Gonabad, Iran. Milk was pasteurized at 75°C for 15 second using a plate pasteurizer and cooled at 5°C. Then milk was transported carefully to a cheese vat with the temperature of 37°C. One lyophilized direct-to-vat mixed culture containing mesophilic (*Streptococcus lactis. lactis* and *Str. lactis. cremoris*) and thermophilic (*Str. thermophilus* and *Lactobacillus bulgaricus*) bacteria, was used at 3 different concentration (1, 2 and 3%). The milk was supplemented with 0.15 g of CaCl₂/kg of milk and held at 35°C until the final pH of milk reached 6, 6.2 and 6.4 before the addition of rennet. As coagulant, chymosin derived by fermentation of *Aspergillus niger* var. *awamori* [standard

rennet, Chy-Max, Chr. Hansen Inc.; 183 International Milk Clotting Units (IMCU)/mL (International Dairy Federation, 1997)] was used at a concentration of 4.5 IMCU/kg of milk. Rennet was diluted 30-fold with cold water then added to each batch of milk.

After approximately 55 min, the curd was cut crossways into cubes of 1 cm³ and left for 10 min. After being cut, the curd was allowed to settle for 3-5 min and then gently agitated at a gradually increasing rate for 10 min to avoid fusion of freshly cut curd cubes and facilitate whey expulsion.

The sliced curd was carefully ladled from the vat into a stainless steel mould of 25 cm (length)×12 cm (width)×10 cm (height) under the initial pressure of 0.3 kPa which gradually increased up to approximately 2.9 kPa at the first hour and held constant to the end of pressing for each lot of cheese. After complete draining, the curd was stored at 16-18°C and covered with 20±1% (w/v) brine (brine was beforehand pasteurized at 80°C for 10 min and filtered through a clean cloth after rapid cooling) for 12 hours. After this stage, the blocks were placed in airtight still containers covered with 10-12% brine and stored at 13-15°C for 4 weeks and kept in 5°C after that.

Sampling: Cheeses were sampled for analysis at the age of 1, 7, 14, 21, 28 and 35 days. One of the cans of cheese was randomly selected and the cheese block was removed and allowed to drip for 2 min. Then, one slice of about 100 g was cut from an edge and another one from the middle of the block and placed in a plastic waterproof bag. The two pieces were ground to a homogeneous paste and small portions were taken from it for chemical analyses in duplicate.

Chemical analyses: Titrable acidity of milk was determined by the Dornic method and its total solids were determined by drying 8-11 g of milk at 100°C for 5 h. The pH of milk and cheese samples was measured using a digital pH-meter (Metrhem, Switzerland). Cheese was analyzed for its total solids by drying 8-11 g of milk at 100°C for 5 h [3] and salt content by Volhard (James) [13].

The fat content of milk and cheese samples was determined by the Gerber method [13] and their total protein contents were determined by measuring total nitrogen using the Kjeldahl method [14] and converting it to protein content by multiplying by 6.38.

Apparent yield was calculated as the weight of cheese before brining (after 19 to 20 h storage at 23 to 25°C) divided by the weight of the milk used.

Experimental procedure: Three concentration of starter culture (1, 2 and 3%) with five levels of pH of renneting (6, 6.2 and 6.4) were evaluated in a randomized experimental design with factorial arrangement. Duncan Multiple-Comparison test ($p < 0.05$) was used to compare means and evaluate simple and interactive effects of treatments. Correlation between variables was analyzed by simple and multivariate regression.

RESULTS AND DISCUSSION

Acidification rate: Acid production at the appropriate rate and time is the key step in the manufacture of a good quality cheese [2]. For this reason, the changes of pH of different cheeses were studied separately during draining as well as during ripening and storage.

Regardless of the concentration of culture used and pH of renneting, the pH of the curd was decreasing during the first day (Fig. 1, 2). After 24 hours in brine, the pH of the curd was around 4.25 for the 3% culture, around 4.4 for 2% culture and around 4.6 for 1% culture. This shows that with increase in concentration of the starter culture

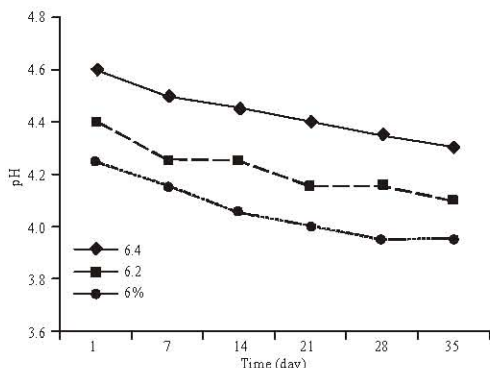


Fig. 1: Effect of starter culture concentration on the pH of Iranian brine cheese during storage

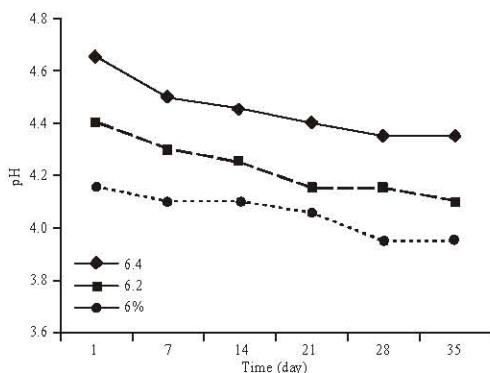


Fig. 2: Effect of renneting pH of milk on the pH of Iranian brine cheese during storage

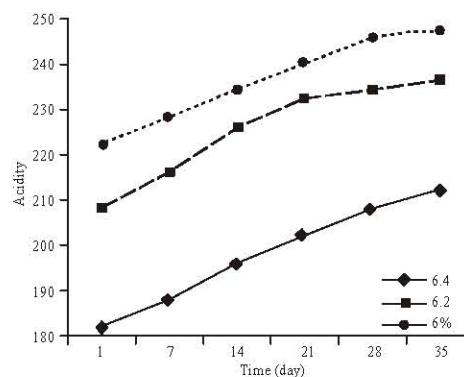


Fig. 3: Effect of starter culture concentration on the acidity of Iranian brine cheese during storage

the pH of cured cheese had a small decrease (Fig. 1). Fig. 2 shows that cheese made with renneting pH of 6 had the lowest pH of all. The most important factor which influences the pH of cured is the pH of milk. With 0.2 decrease in pH of raw milk, the pH of cured will be less than 4.35. Although the pH of cured made of milk with pH = 6 is not suitable and is very low, but cheese made with pH=6.2-6.4 is proper. Although not statistically supported, similar results were reported by Litopoulou *et al.* [15] and Pappa and Anyfantakis [16] for Feta cheese and Kehagias *et al.* [17] for Teleme and Feta cheeses.

Figure 1 and 2 show that the pH in cheeses was still decreasing, until transferred to cold room, regardless of the concentration of starter culture and pH of renneting. Nevertheless, the rate of pH decrease was higher in cheese made from 1% starter culture and renneting pH of 6.4. After 35 days of storage, the pH of the curd was around 3.95 for the 3% culture, around 4.1 for 2% culture and around 4.3 for 1% culture.

Similar to our results, Azarnia *et al.* [18] reported a decrease in pH during ripening of IWC in brine (10% wt/vol, pH = 7.4), mainly because of completion of lactose fermentation and the liberation of amino and free fatty acids. The slow solubilization of colloidal calcium phosphate during ripening, which causes a slow increase in pH [19], may have offset the tendency for a pH decrease in the present study, with the result that the cheese pH remained stable.

The variation of acidity of cheeses made with different content of starter culture during storage is depicted in Fig. 3. It can be seen that the acidity of cheeses increased with the increase in starter culture content during storage. Decrease in renneting pH of milk resulted lower acidity in final cheeses (Fig. 4). Cheeses made with milk with higher pH had the lowest acidity. Acidity of all cheeses increased during storage period.

Table 1: Changes of NaCl and total solid of cheeses made with different concentration and renneting pH of milk during ripening and storage

Age, day	pH	NaCl (%)			Total solid (%)		
		Lactic acid concentration (%)			Lactic acid concentration (%)		
		1	2	3	1	2	3
1	6.4	3.6	3.3	2.6	40.0	40.0	41.0
	6.2	3.3	3.6	3.6	40.0	38.0	37.0
	6.0	3.6	4.0	4.3	38.0	36.6	36.3
7	6.4	3.8	3.5	2.8	41.0	40.8	41.3
	6.2	3.7	4.0	4.3	40.4	38.9	38.1
	6.0	4.1	4.5	4.9	38.8	37.8	37.8
14	6.4	3.9	3.6	3.0	41.8	41.2	41.5
	6.2	4.0	4.3	4.6	40.8	39.8	39.2
	6.0	4.6	4.9	5.4	39.5	39.0	38.7
21	6.4	3.9	3.7	3.2	42.3	41.8	41.9
	6.2	4.2	4.6	5.0	41.3	40.5	40.3
	6.0	5.0	5.3	5.8	40.5	40.2	39.9
28	6.4	4.0	3.8	3.3	42.8	42.4	42.3
	6.2	4.3	4.8	5.5	41.7	41.4	41.4
	6.0	5.3	5.5	6.1	41.5	41.5	41.2
35	6.4	4.0	3.8	3.3	43.0	42.7	42.5
	6.2	4.4	4.9	5.7	42.0	41.8	41.8
	6.0	5.4	5.7	6.3	41.9	41.8	41.7

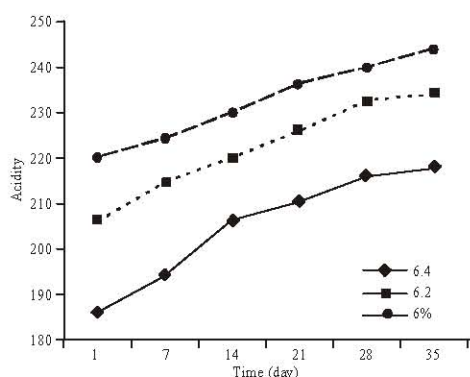


Fig. 4: Effect of renneting pH of milk on the acidity of Iranian brine cheese during storage

The value of pH at renneting significantly influenced the chemical characteristics of the treatments. As the acidification rate enhanced by increasing the concentration of inoculated starter to milk, the ash content of the treatments will decrease, whereas their protein content increases [20].

Changes in NaCl content: Table 1 show that the NaCl content of all cheeses ranged from 2.6% to 4.3% on day 1 and increased from 2.8% to 6.3% after storage. From the statistical analysis it is observed that the NaCl content of Iranian cheese was increasing until 28 days of storage and

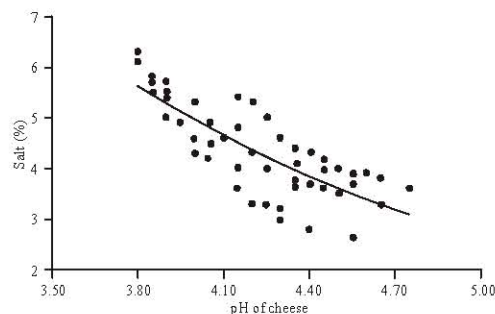


Fig. 5: Relationship between salt (%) and final pH of cheese

generally, remained stable thereafter, without significant differences ($P>0.05$). Among the cheeses, cheese made with 3% starter culture and milk pH of 6 had the highest NaCl of all during storage period.

This was an expected result because the cheese had high moisture content on day 1 and the excess of it was drained off by syneresis, pH development, salting and ripening changes. While moisture is being expelled from the cheese into brine, salt is diffused from brine into the cheese until equilibrium is achieved. With increase in concentration of lactic starter and decrease in pH of renneting, the salt content in cheese increased exponentially (Fig. 5). This shows that with increase in starter concentration and decrease in pH of milk

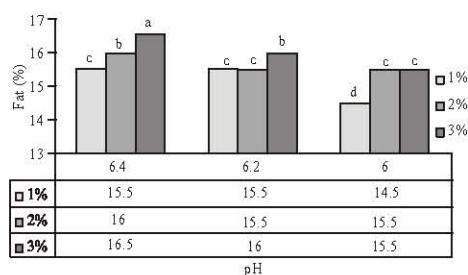


Fig. 6: Effect of starter culture concentration and renneting pH of milk on fat content of Iranian brine cheese

before renneting the salt absorption will increase in cheese during storage. When cheese is placed in brine, there is a net movement of NaCl molecules, as Na⁺ and Cl⁻, from the brine into the cheese because of the osmotic pressure difference between the cheese moisture and the brine. Consequently, the water in the cheese containing dissolved materials such as acids and minerals (calcium, magnesium, phosphorus, potassium, etc.) diffuses out through the cheese matrix with a flux approximately twice that of NaCl so as to restore osmotic pressure equilibrium [21]. The establishment of these dynamic phenomena decreased the moisture content of the treatments and increased their salt content as they ripened.

Zerfiridis *et al.* [22] and Papa *et al.* [5] applying brine and dry salting of Teleme cheese made from cows' milk reported also that the NaCl content was stabilized at the time of transfer to cold room, regardless of the kind of culture used. Dagdemir *et al.* [23] also found that the starter cultures made no difference in the NaCl content of a Turkish white cheese.

Changes in fat content: As starter concentration (%) increased from 1 to 3% (w.b.), fat content increased slightly from 15.5 to 16.5%, 15.5 to 16% and 14.5 to 15.5% for cheese made with renneting pH of 6.4, 6.5 and 6 on day one, respectively (Fig. 6). Although increase in content of starter culture had an small effect on fat content but this increase was not significant. With decrease in renneting pH of milk fat content decreased but this decrease was also not significant. The estimated fat content of Iranian brine cheese with starter culture was lower than Telme cheese [5]. It is probable that milk being richer in casein and fat gives a better coagulation, more firm coagulum and less loss of fat in the whey. Thus, cheese made with higher starter culture and renneting pH has the better acceptability. The effect of addition of starter culture on fat content of cheese has

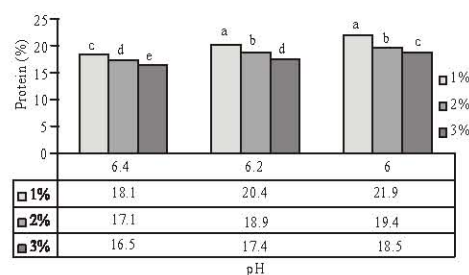


Fig. 7: Effect of starter culture concentration and renneting pH of milk on protein content of Iranian brine cheese

been evidenced in other findings. Khosroshahi *et al.* [20] reported that all of the measured attributes for Iranian white cheese except fat content were altered by changing the starter concentration or by aging, or both, at 5°C. Watkinson *et al.* [24] also reported no change for fat area distribution with pH and storage time for semihard model cheeses.

Changes in protein: As it is seen in Fig. 7, the protein content of the fresh cheeses increased with the increase in starter culture content. The results also showed that protein of Iranian brine cheese with different pH of renneting ranged from 18.9 to 21.9% for cheese made with 3% starter culture, 17.1 to 19.4% for cheese with 2% starter culture and 16.5 to 18.5% for cheese with 1% culture. The protein of cheese increased with increase in pH of cheese milk.

It is obvious that the thermophilic starter available in the starter culture, being more proteolytic than the mesophilic starters, caused a higher decrease of proteins in cheeses. Thus, with increase in the content of this starter culture the amount of protein decreases. The differences in protein content of cheeses during ripening are due to hydrolysis of proteins to water soluble nitrogenous compounds and to the diffusion of these products into the brine [25]. Also, the high degree of hydrolysis contributes to the protein decrease as the migration of such compounds of cheese into brine is determined by factors such as size and hydrophobicity of the water soluble nitrogenous compounds [26].

Lower values of pH at renneting reduced the net charge on casein micelles and probably improved the activity of rennet [27], leading to greater protein recovery in the curd. This, therefore, increased the protein content of cheese treatments. In agreement with the result obtained in the present study, Banks *et al.* [12] reported that the amount of CP in cheese obtained from normally

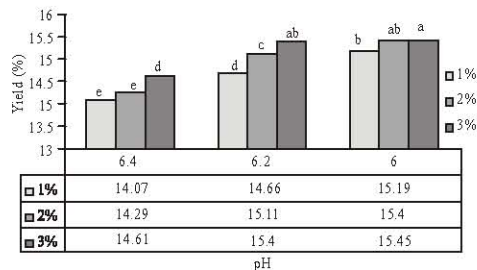


Fig. 8: Effect of starter culture concentration and renneting pH of milk on yield of Iranian brine cheese

heat-treated milk (72°C for 16 s) acidified to pH 5.8 was higher than that of unacidified milk.

Change in total solid and yield: The effect of content of starter pH of renneting of Iranian brine cheeses, at each different age is shown in Table 1. With decrease in pH of renneting, the total solid of cheese decrease in all starter content and storage time. Increasing of starter culture content had no significant effect on total solid of cured and stored cheese. Total solid of Iranian brine cheese increased during storage time.

From the statistical analysis of yield of cheeses made with different starter culture and pH of renneting it is evident that with increase in starter content and decrease in renneting pH, cheese yield increases significantly ($P < 0.05$) during the early days of cheese manufacture (Fig. 8). The changes in yield can be attributed to changes in moisture content. Feta cheese made from ewes' milk and a thermophilic culture by Pappa *et al.* [5] was found to have higher yield than that in this study for Teleme cheese. Similar results to this study were found by Pappa and Anyfantakis [16] for mature Feta cheese made with a mesophilic and with a mixed mesophilic-thermophilic culture and Mallatou *et al.* [28] for Feta cheese made with a thermophilic culture. Zerfiridis *et al.* [22] reported lower values for mature (60-120 days) Feta cheese made from ewes' milk. Concerning Teleme cheese made from goats-milk, Zerfiridis *et al.* [22] and Zygouris [29] reported yields similar to those in this study, whereas Mallatou *et al.* [28] found lower values for a white-brined cheese.

Final pH of cheese had correlation with yield (%). Regression analysis between pH of cheese and yield of cheese is shown in Fig. 9. With increase in final pH of cheese up to 4.4, a small decrease in yield was observed and afterwards, the value increased sharply. Thus, the addition of starter culture and increase its concentration which leads to decrease in pH causes an increase in yield

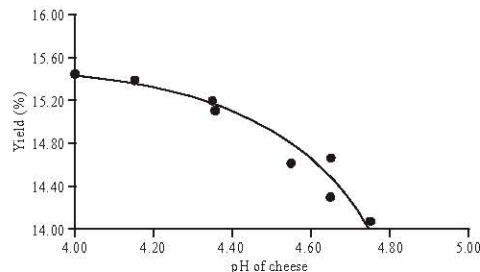


Fig. 9: Relationship between yield (%) and final pH of cheese

content. Decrease in pH of renneting of milk which also decreases the final pH of cheese results in increase of cheese yield.

CONCLUSIONS

The rate of pH decrease was the fastest in cheeses made with 3% starter culture and renneting pH of 6 and the slowest in cheeses made with the 1% culture and renneting pH of 6.4. The acidity was higher in cheese made with 3% starter culture. The highest fat content was in cheeses made with 3% starter culture and renneting pH of 6.4 and the highest yield in fresh cheese was in cheese made with 3% culture and renneting pH of 6. Starter culture concentration and renneting pH had significant impact on salt content in cheese where as total solid was not affected by starter culture concentration. During ripening, culture concentration and pH of renneting significantly affected salt content in cheese.

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