Effect of Different Concentrations of Rennet on Some Parameters of White Brine Cheese

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**ABSTRACT**

This work was conducted to study of influence of fungal rennet concentration on some traits including physico-chemical (Dry matter, Fat, Salt, pH, acidity and protein)% and proteolysis (WSN, NPN)% of white brine cheese. Effect of ripening period on both physicochemical and proteolysis was not significant (P>0.05). Among some physicochemical properties and proteolysis indices, dry matter%, protein% and pH decreased but fat%, salt%, acidity%, WSN% and NPN% increased until the end of ripening period. The results showed that cheeses made with 2% fungal rennet had highest yield among all cheeses.

**INTRODUCTION**

Cheese making is a process concentrating milk components, in particular fat and protein contents which are deterrent factors of cheese yield [1]. Cheese yield is vital in an economic sense for cheese makers since small differences in yield translate into big differences in profits [2]. Cheese yield is defined as the amount of cheese, expressed in kilograms, obtained from 100 kg of milk [3]. It is a very important parameter: The higher the recovered percentage of Solids, The greater is the amount of cheese obtained and therefore gains in economic terms. It is, therefore, obvious how to elaborate a rapid method that allows for an estimate, before transformation, of the find cheese yield on the basis of the composition of the raw material [1].

White brined cheese, like other types of ripened cheese, required maturation to develop the required properties. Pickled cheeses are in great demand in warm climates and their preservation in brine is necessary. The specific characteristics of brine cheese develop in the salted water or whey and the chemical, physical and biochemical properties of this type of cheese are controlled and often restricted by the environmental conditions [4]. The efficiency is a property of rennet and is called the strength of coagulation. This denotes the amount of cm³ of fresh milk of a certain acidity that, at the temperature of 35°C, will coagulate into a solid gel after the addition of 1 cm³ of liquid or 1 g of solid rennet during less than 60 min. An increase in the concentration of rennet caused gradual growth of viscosity and density of coagulate and a decrease of the rate of the separation of whey. For this reason, it is important to monitor and optimize the process of cheese production and thus obtain products of a high value [5].

**MATERIAL AND METHODS**

Cheese making:

Ewe’s milk from the Zandy breed was supplied from a farm in Varamin. Experimental cheese samples were made in three replications at the Tehran Pegah dairy plant (Tehran, Iran). Lighvan cheese was produced using raw milk. The raw milk was warmed to 36°C, and coagulated with microbial rennet for 60 minutes. After curdling, the curd was cut into cubes of approximately 1 cm³ and left to rest for 15 minutes. The slab curd was placed on a mesh table and weighted for draining. After whey separation was completed, the curd was cut into large cubes (approximately 10x10x7 cm³) and immersed in brine with 22% concentration for about seven hours at room temperature. The cheese blocks were placed into a tin-plate container with brine salted to about 12% concentration. The container was sealed and stored for 90 days [6].

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Chemical analysis:
Samples of cheese were analyzed for pH (Metrohm Model 632 pH-meter; Switzerland) and percentages of titratable acidity, protein, dry matter, ash, total fat and salt after 7, 30, 60 and 90 days of ripening (AOAC, 2000).

Nitrogen Fractionation:
Water-soluble N (WSN) and N soluble in 12% tri-chloroacetic acid (TCA-SN) were determined in aliquots of water-soluble extract (WSE) prepared as described by authors (13), except that the cheese: water ratio was 1:5, a Sorvall Omni-mixer (Dupont Company, Newton, CT, USA.) was used for homogenization and the supernatant obtained was filtered through Whatman No. 42 filter paper. The TCA-SN fraction was obtained by mixing 10 ml of WSE with 10 ml of 24% (w/v) aqueous solution of TCA, holding the mixture at room temperature for 1 hour and then filtering it through Whatman No. 42 filter paper [7].

Statistical analysis
The data were statistically analyzed using a completely randomized design (CRD) with three replications. Data were subjected to analysis of variance using the SAS statistical software package (SAS, 1988). Mean comparison was performed with LSD’s test at the P < 0.05 level of significance.

RESULT AND DISCUSSION

As shown in table 1, Physicochemical properties of white brine cheese (pH, Acidity, Dry matter, Protein, Salt, ash and Fat) mg/100 g of sample had not significant (P>0.05) differences among all treatments during ripening period (90 days). Dry matter content (g/100g) of all treatments decreased during ripening period. Highest content of dry matter was attributed to treatment which made from 2% fungal rennet at 90 days of ripening. Decrease in dry matter was attributed to water–soluble proteins and peptides passing from the cheese matrix to the brine; really, this decrease may be due mainly to the breaking of peptide bonds and the release of new ionic groups [6]. Other researchers reported that the dry matter content of Urfa cheese decreased throughout storage as a result of extended proteolysis [8].

The total fat content (g/100g) of the white brine cheese at the start of ripening was 17.52 g/100g; this decreased to 15.62 g/100g during ripening. Changes in fat content could be due to a decrease in dry matter [6]. In this matter, lipolytic activity of microflora on fat results in leakage of some fat from the curd into brining whey [9]. Salt content (g/100g) of the white brine cheese at the start of ripening was 2.78 g/100g; this increased to 5.03(g/100g) by the end of ripening. Salt is driven into cheese by the concentration gradient between the cheese blocks and brine; this gradient is much larger at the beginning of ripening [4].

Acid production at the appropriate rate and time is the key step in the manufacture of a good quality cheese. Regardless of the concentration of fungal rennet and pH of renneting, the pH of the curd was decreasing during ripening period. The pH content of the white brine cheese at the start of ripening was 5.27. This decreased to 5.01 until the end of ripening time. This shows that with increasing in concentration of fungal rennet, the pH of curd cheese had a small decrease. The most important factor which influences the pH curd is the pH of milk. Nevertheless, the rate of decreasing of pH was higher in cheese made from 3% fungal rennet. Parallel to loss of pH of all treatments, acidity of all samples increased during ripening, acidity content (g/100g) of the white brine cheese at the start of ripening was 0.68(g/100g); this increased to 0.85(g/100g) during ripening, the increase in acidity mainly due to completion of lactose fermentation and the liberation of amino and free fatty acids [4].
The slow solubilization of colloidal calcium phosphate during ripening, which causes a slow increase in pH [10]. Water Soluble Nitrogen (WSN) content (g/100g) of the white brine cheese at the start of ripening was 0.62(g/100g); this increased to 0.88 (g/100g) during ripening period and also Non-protein Nitrogen (NPN) content (g/100g) of the white brine cheese at the start of ripening was 0.13(g/100g); this increased to 0.30(g/100g) at the end of ripening period. Increase in WSN and NPN of treatment which had 2 and 3% of fungal rennet were higher than treatment with 1% fungal rennet.

Of course, there were small differences between samples at the same ripening period. Different levels of rennet had not significantly (P>0.05) affected cheese proteolysis in white brine cheese and no regular changes were noted among the cheeses as dependent of rennet concentrations in terms of WSN or NPN contents. A similar trend was found by Kubis et al., [11], who reported that, the pH 4.6. Soluble nitrogen contents of Cheddar-type goat’s milk cheeses increased during ripening, and its level was generally proportional to rennet levels. The protein (g/100g) decreased from 14.42 to 13.88(g/100g) during ripening of white brine cheese because of proteolysis, the amount of protein decreases during ripening, releasing amino acids that are transformed into volatile compounds. The differences in protein content of the cheeses during ripening are attributed to hydrolysis of proteins to WSN compounds and to the diffusion of these products into brine. 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 [6].

NPX is known to be an indication of the amount of small peptides and amino acids present in cheese, and its level is considered to serve as an index of ripening depth and extending the ripening time leads to an increase in protein degradation and production of NPN in cheese [6].

**Table1:** Physicochemical parameters and proteolysis indices of white brine cheese with different concentration of rennet.

<table>
<thead>
<tr>
<th>Days of ripening</th>
<th>Concentration of rennet(%)</th>
<th>Physicochemical parameters (g/100g)</th>
<th>Proteolysis indices (g/100g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low (&lt;0.2%)</td>
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<tr>
<td></td>
<td></td>
<td>Medium (0.2-0.35)</td>
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<td></td>
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<td>High (&gt;0.35)</td>
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<tr>
<td></td>
<td></td>
<td>Dry matter</td>
<td>pH</td>
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<tr>
<td>7</td>
<td>1</td>
<td>43.9</td>
<td>5.28</td>
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<tr>
<td>30</td>
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<td>42.76</td>
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<tr>
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<td>60</td>
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<tr>
<td></td>
<td>3</td>
<td>40.12</td>
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</table>

Means in each row without a superscript did not differ significantly (P>0.05).

From the statistical analysis of yield of cheeses made with different concentrations of fungal rennet, It is obvious that with increase in rennet concentration up to 2%, cheese yield increases significantly (P<0.05) during the early days of cheese manufacture (Figure, 2). The changes in yield can be attributed to changes in moisture content also fat %, protein % and salt% have an important role in cheese yield. Similar result was reported by Guven et al., [12] who studied influence of rennet concentration on ripening characteristics of Halloumi cheese.

**Fig. 2:** Cheese yield with different concentration of fungal rennet.
Conclusion:
Coagulation level did not affected significantly ($P>0.05$). The gross composition of white brine cheese; thus, the cheese obtained with 2% rennet level showed a lower moisture content than the others. No significant ($P>0.05$) differences were determined between the cheese manufactured with different levels of rennet in terms of soluble nitrogen fractions and non-protein nitrogen. It was concluded that when rennet concentration increased up to 2% of milk used in cheese-making, yield cheese also increased and improved the gross-composition of white brine cheese.

REFERENCES