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Effect of Animal and Microbial Rennet on Proteolysis During Ripening of White Brined Cheese

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Abstract. The objective of this research was to summarize the effect of rennet type (calf rennet and microbial rennet from *Rhizomucor miehei*) and starter culture on the biochemical properties and free amino acid (FAA) release during the ripening of Turkish White brined cheese. The concentrations of FAAs and physiochemical characteristics were similar for cheeses made with both types of coagulant. Phe, Leu - Ile, Gln, Val, Pro and Ala were the principal FAAs in the White brined cheeses at all stages of ripening.

Keywords: White Brined Cheese, Rennet, Starter Culture, Proteolysis

1. Introduction

Proteolysis is usually regarded as the main biochemical process during cheese ripening and one of the most important factors for the development of typical cheese flavor and texture. Rennet or a similar coagulating enzyme, plasmin, the starter bacteria, and the non-starter microflora are the main proteolytic agents involved in cheese ripening. Proteins are partially hydrolyzed by rennet and other native microbial enzymes to produce lower-molecular-weight compounds and are further broken down by peptidases into various nitrogenous substances, such as proteose, peptone, amino acids and amines (Farkye and Fox, 1990; Fox and McSweeney 1996; Fox et al. 1999; Martinez-Cuesta et al. 2001).

Turkish White cheese, produced traditionally in nearly every part of the country, is the most popular cheese variety in Turkey, representing approximately 60% of the country's total cheese production. This cheese was originally manufactured from sheep or goat's milk, but cow's milk or a combination of milks is now generally used for its production. White Cheese is a cheese variety that is brine-salted and ripened in brine (a 12-14 g/100 g NaCl solution). A typical White Cheese has its own characteristic flavor, soft or semi-hard texture, and very fine or no eyes. It has a salty, acid taste, is suitable for slicing and is liable to crumble. The cheese is matured for a period of 1-3 months (Dinkci and Gonc, 2000; Hayaloglu et al. 2002; Kamber 2008).

Coagulating enzymes are essential ingredients for the production of ripened cheese varieties. Rennet is a milk coagulating enzymatic preparation. The most highly active enzyme in animal rennet is chymosin. In cheese making, the principal role of chymosin, is to coagulate milk by specific hydrolysis of Phe105-Met106 bond of the *k*casein (micelle-stabilising protein), causing milk coagulation (Fox et al. 2000).

Commercial calf rennet is used in White Cheese manufacturing primarily as a milk coagulant. However, the increased consumption of cheese has led to an increase in the demand for rennet, while there has been a decrease in the number of young animals (leading to the investigation of alternative milk clothing enzymes of different origins). Therefore, other suitable coagulants (bovine, porcine and chicken), including proteinases from microorganisms (*Mucor miehei*, *Mucor pucillus* and *Cryphonectria parasitica* (formerly

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Endothia parasitica)), have become more popular in the production of cheeses (Jacob et al. 2010). Therefore, the objectives of this study were to investigate the effects of different rennet types (calf and microbial from *Rhizomucor miehei*) together with no addition or addition of the starter on the some physiochemical characteristics and free amino acid (FAA) content of whole-fat Turkish White brined cheese.

2. Materials and Methods

2.1. Experimental Design

White brined cheeses were produced by the traditional method Hayaloglu et al. (2002). The cheese-making trials were denoted as A, A5, A10, A20, M, M5, M10 and M20. The experimental design of white brined cheeses is detailed in Table 1.

2.2. Methods

The following analyses were performed on the cheese samples: pH (internal and external) was determined with a potentiometer; titratable acidity (SH: Soxhlet Henkel) was determined by titrating 10 g of sample with 0.25 N NaOH to a pink endpoint using a phenolphthalein indicator (Kurt et al. 2006); formal titration (FT) was determined according to the Schilowitsch method modified by Scancalapore ve Longone (1988). The N content, both the total nitrogen (TN) and the N content of the nitrogenous fraction [water soluble nitrogen (WSN) and nitrogen soluble in 12% trichloroacetic acid (TCA-SN)], were determined by the Kjeldahl method (IDF, 1993). A ripening index (RI) related to rennet activity was determined by the methods of Eren-Vapur (2010). The free amino acid (FAA) content was employed in the determination of proteolysis using a LC/MS according to the methods of Ozcan and Senyuva (2006). All chemical measurements were done in triplicate. Cheese samples were chemically analyzed at the 90 days of ripening.

Table 1: Experimental design of White brined cheeses

Cheese trials	Culture[a]	Animal rennet[b]	Microbial rennet[c]
A	–[d]	+	–
A_5	+	+	–
A_{10}	+	+	–
A_{20}	+	+	–
M	–	–	+
M_5	+	–	+
M_{10}	+	–	+
A_{20}	+	–	+

[a] Starter culture, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp *cremoris*. *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*, 5 DCU/tons, 10 DCU/tons and 20 DCU/tons (DCU: Danisco Culture Unit: Danisco, Brugge, Belgium)
[b] Animal rennet (1/18 000: Mayasan Food Industries A.S. Istanbul, Turkey)
[c] Microbial rennet (1/16 000: Mayasan Food Industries A.S. Istanbul, Turkey)
[d] +, addition; −, no addition.

3. Results and Discussion

The physiochemical properties of White brined cheeses are presented in Table 2. White cheese ripening and proteolysis were influenced by starter cultures and coagulants ($P<0.05$, $P<0.01$). The rates of proteolysis in terms of total nitrogen (TN), water-soluble nitrogen (WSN) and ripening index (RI) were affected by starter culture and ripening time. The milk clotting enzymes contribute to proteolysis in Turkish White cheese. This is due to the high coagulant retention of the cheese curd with high moisture content, and also due to storage of cheese in salted whey that contains residual coagulant. The use of calf rennet and microbial rennets (e.g., *R. miehei*) and their implication on proteolysis during cheese production and ripening have been demonstrated by Yesilyurt (1992), Uysal (1996), Saldamli and Kaytanli (1998) and Yetismeyen et al. (1998).

The average free amino acid (FAA) composition of White brined cheeses is presented in Table 3. The FAAs of the cheeses were significantly affected by the addition of a different source of enzymes, the starter culture and the ripening period (P<0.01). Proteolysis in Turkish White cheese continues during storage in brine. Starter peptidases are responsible for the production of amino acids. The addition of lactic acid bacteria (LAB) as a starter culture produced a higher content of short-chain peptides and FAAs during cheese ripening (Lee et al. 1990; Lane and Fox, 1996). However, different starter bacteria release different levels of individual FAA (Dr ăb et al. 1999) based on their enzyme system and the degree of autolysis in the cheese (Brome and Limsowtin, 1998). The total concentration of FAAs increased during ripening and Phe, Leu - Ile, Gln, Pro, Ala and Val were the principal FAAs in the cheeses at all stages of ripening (Table 3).

The principal FAAs, including Leu, Glu, Phe, Val and Lys, were present in the 60-day old Turkish White brined cheese made from pasteurized cow's milk (Ucuncu, 1981; Kaymaz, 1982; Hayaloğlu et al. 2004). Previous authors (Alichanidis et al. 1984; Katsiari et al. 2000; Michaelidou et al. 2003) have shown that Leu, Glu, Val and Lys were major FAAs in Feta cheese made from cow's milk. However, while the same FAAs were present in Iranian Brine cheeses after ripening for 50 days, this did not continue to the end of ripening in Feta; the amino acid Lys, Arg and Glu were predominant at the end of ripening in the cheese (Azarnia et al. 1997). According to these authors, the decrease in FAA could be due to amino acid catabolism, or as explained by Caric (1987), the amino acids diffused into the brine.

Table 2: The changes in physiochemical properties of White brined cheeses during ripening

	N	TN (%)	WSN (%)	TCA (%)	Rip. Index (%)	Formal Tit. (%)	Acidity (SH)	pH (Int.)	pH (Ext.)
Rennet									
A	80	2,60[a]	0,48[a]	0,19[a]	28,49[a]	0,28[a]	76,49[a]	4,87[a]	4,91[a]
M	80	2,66[a]	0,43[a]	0,19[a]	27,61[a]	0,26[a]	77,20[a]	4,84[a]	4,89[a]
Sx		±0,02	±0,07	±0,005	±0,46	±0,004	±0,69	±0,010	±0,01
Starter Culture									
Control	40	2,69[a]	0,52[a]	0,19[a]	29,49[a]	0,29[a]	59,665[c]	5,09[a]	5,13[a]
5	40	2,56[c]	0,44[b]	0,18[a]	29,56[a]	0,26[bc]	79,246[b]	4,78[b]	4,82[b]
10	40	2,60[ab]	0,42[b]	0,20[a]	27,01[b]	0,25[c]	83,196[a]	4,78[b]	4,83[b]
20	40	2,66[b]	0,44[b]	0,19[a]	26,13[b]	0,27[a]	85,278[a]	4,76[b]	4,80[b]
Sx		±0,28	±0,01	±0,002	±0,64	±0,005	±0,969	±0,02	±0,01
Ripening Time (d)									
1	32	3,41[a]	0,25[c]	0,11[d]	10,018[d]	0,17[e]	82,86[a]	4,85[ab]	4,88[b]
15	32	2,62[b]	0,36[d]	0,13[d]	22,890[c]	0,22[d]	71,24[a]	4,87[a]	4,95[a]
30	32	2,56[b]	0,45[d]	0,19[c]	23,453[c]	0,28[b]	69,37[c]	4,86[ab]	4,91[b]
60	32	2,34[c]	0,54[b]	0,23[b]	36,218[b]	0,32[c]	77,88[b]	4,88[a]	4,91[b]
90	32	2,22[d]	0,66[a]	0,29[a]	38,656[a]	0,36[a]	82,94[a]	4,81[a]	4,83[c]
Sx		±0,31	±0,01	±0,008	±0,723	±0,006	±1,084	±0,02	±0,01
Rennet		*	**	ns	ns	**	ns	ns	ns
Culture		**	**	ns	**	**	**	**	**
Time		**	**	**	**	**	**	ns	**

Significance level: significant at p<0.01 (**), p<0.05 (*), different superscript letters on the same column indicate significant differences, ns: non significan

A: Animal rennet, A5: Animal rennet+5DCU starter culture, A10: Animal rennet+10DCU starter culture, A20: Animal rennet+20DCU starter culture, M: Microbial rennet, M5: Microbial rennet+5DCU starter culture, M10: Microbial rennet+10DCU starter culture, M20: Microbial rennet+20DCU starter culture

4. Conclusion

Many studies have focused on the proteolytic activity of enzymes in rennet during cheese making, and FAA content is often used as an index for cheese maturity. Ongoing research in this area will produce a much clearer picture of the role of these enzymes in cheese ripening.

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Table 3: The free amino acid (FAA) composition of White brined cheeses (mg 100 g-1 of cheese dry matter)

Amino Acid	**A**	**A5**	**A10**	**A20**	**M**	**M5**	**M10**	**M20**
Asparagine (Asn)	0.910	0.930	0.51	0.585	0.625	0.670	0.905	0.625
Aspartic acid (Asp)	0.120	0.110	0.05	<LOD	0.120	0.120	0.125	0.120
Serine (Ser)	0.165	0.200	0.165	0.200	0.100	0.215	0.195	0.200
Glycine (Gly)	0.245	0.280	0.165	0.250	0.115	0.270	0.220	0.185
Glutamine (Gln)	1.895	1.945	1.160	1.890	0.610	1.970	1.360	1.840
Lysine (Lys)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cysteine (Cys-Cys)	0.260	0.185	0.155	0.160	0.145	0.155	0.21	0.245
Glutamic acid (Glu)	0.615	0.615	0.555	0.540	0.375	0.495	0.525	0.533
Threonine (Thr)	0.195	0.245	0.185	0.190	0.110	0.265	0.230	0.215
Alanine (Ala)	2.065	1.655	0.895	1.270	0.785	2.020	1.295	1.585
Proline (Pro)	2.350	2.250	1.31	2.070	1.270	2.745	2.065	2.080
Valine (Val)	3.975	2.635	1.385	1.610	2.205	4.075	2.375	2.005
Methionine (Met)	0.415	0.580	0.095	0.370	0.250	0.720	0.620	0.655
Tryptophan (Trp)	0.070	0.060	<LOD	0.100	<LOD	0.030	0.020	0.070
Arginine (Arg)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cysteine (Cys)	0.075	0.075	0.415	0.650	0.520	0.115	0.065	0.445
Tyrosine (Tyr)	0.035	0.025	0.01	0.190	0.010	0.030	0.015	0.030
Phenylalanine (Phe)	6.850	6.155	3.525	4.650	3.725	8.450	4.270	4.595
Hydroxyproline (Hyp)	0.290	0.210	0.190	0.180	0.100	0.270	0.185	1.175
Leucine- Isoleucine (Leu - Ile)	7.880	4.785	2.845	3.885	1.975	6.7000	3.330	3.105
Histidine (His)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Total	28.410	22.940	13.615	18.790	13.040	29.310	18.010	19.708

A: Animal rennet. A5: Animal rennet+5DCU starter culture. A10: Animal rennet+10DCU starter culture. A20: Animal rennet+20DCU starter culture. M: Microbial rennet. M5: Microbial rennet+5DCU starter culture. M10: Microbial rennet+10DCU starter culture. M20: Microbial rennet+20DCU starter culture

LOD: limit of detection

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