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RESEARCH ARTICLE

RESEARCH STUDIES ON CHEESE BRINE RIPENING

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Abstract:

The research is focused on cheese maturation as a consequence of several biochemical and microbiological phenomena, all of which involve great changes of milk components. Among these lactose fermentation, proteolyses, lyses of fats, transformations of amino acids as will as generation of volatile compounds are more important then others. Therefore, as a result of such phenomena cheese specific flavour and some sensorial and rheological proprieties can be damaged.

Keywords: brine cheese, proteolysis, lactose, nitrogenous fractions.

1. Introduction

Grade milk and dairy products bear, the stamp of a significant position in people's rational diet being at the same time, on account of their chemical composition and assimilation, one of the most accessible animal origin protein sources.

Nutritional interest in such food results, is generally associated with the presence in its composition of the great biological value protein, calcium, in general at rates relatively great, the same as the great biological value of phosphorus and vitamins A and D in especially.

„Telemea" cheese becomes thus a priority consumption and representing 60% of all kinds of cheeses in Romania. The quality of cheese depends on a variety of factors among which raw milk composition, technological process parameters, bacteria species, storage, transportation and delivery conditions (Pappa *et al.*, 2007). The use of started cultures in cheese making practice bearing their impact upon applied biotechnology makes cheese one of the most complex and dynamic foods (El Soda, 1993).

They contribute to sensory characteristic improvement and a higher rate of specific flavours. Each piece in turn can be considered a bioreactor as many complex reactions take place there, in while the final product presents particular sensory characteristics (Costin, 2003). The complexity of the

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biotechnological process in cheese making practice is characterized by several physical, chemical, biochemical and microbiological transformations.

2. Materials and methods

The research was done on brine cheese manufactured according to the classical method from Braila Stancuta cow milk bearing the following characteristics: 1.029 kg/m^3 density, 19 °T tartness, 6.6 pH and 3% fats.

Milk was coagulated with Fromase–Chr.Hansen 22000TL from *Rhizomucor miehei* with coagulation power P_c = 1:150.000. For the Telemea cheese a DVS starter culture–Danisco EZAL France CHOOZITMT1LYO10DC1 was used. This starter consists of: *Lactococcus lactis*, *Lactococcus cremoris*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

The final product was subject for analysis in a technological process following the steps of pressing, salting and ripening from to 40 days period.

The following determinations were done for dry substance, salt content, lactose, pH, titration acidity. The proteolysis that occurs during ripening was monitored from nitrogenous fraction analysis with fractionated precipitation and the extract obtained by Kjeldahl methods. The evolution of proteolysis was studied as the principal nitrous factions considered at different stages of the ripening process and it was quantified by a series of indicators of the ripening statute.

3. Results and discussion

3.1.The evolution of the dry substance

The dry substances content depending on the primary cheese content of water, pH and the brine concentration increases for the brine cheese.

This increase rise to 5-10% (Abd El Salam,1993). The values for dry substance over the brine cheese as a final product are shown in Table 1.

Table 1. *The dry substance and salt contain evolution along the brine cheese obtaining process*

The fabrication step		**Dry substance % cheese**	**NaCl**	
			% cheese	**% dry substance**
after the pressing		36,96	0	0
after the salting		39,33	2,25	5,72
maturation, days	5	40,59	3,35	8,22
	10	41,35	3,40	8,23
	15	41,37	3,47	8,39
	20	41,39	3,50	8,48
	25	41,40	3,52	8,50
	30	41,41	3,53	8,52
	35	41,42	3,55	8,57
	40	41,42	3,55	8,57

The soft cheese contains a rate of 36.96% dry substance immediately after the pressing stage. Consequently, a difference arises between the brine's osmotic pressure and that of the liquid it generates leading to the double diffusion process: the transition of the liquid phase from the cheese concurrent with the salt migration from the brine (Guinee and Fox, 2004). Thus the dry substance after the pressing increases with 6.4% in reference with the no pressing cheese, getting around 39.33%.

The dry substance content is continuously increasing in the first 10 days by 5%, as compared to the maturation beginning and reaches 41.35% all along. Thus, after 35 days of maturation the diffusion process has seriously come down while the dry

substance contained in cheese reached 41.42%, of a rate constantly maintained.

After 40 days of maturation the dry substance content in brine increases by 5.33% as compared to the beginning of maturation and by 11.7% over the pressed cheese. These results are in correspondence with those reported by Abd El Salam (1993) and Costin and Rotaru (1970).

3.2. The salt concentration from the brine cheese

The salt concentration in cheese depends on its former rate as soft cheese, the gradient rate of salt in brine during cheese liquid stage, kind of salt, temperature of cheese and pH (Pavia *et al*., 2000).

After pressing, soft cheese is introduced in brine solution. As a consequence of the osmotic pressure differences a slow NaCl molecule motion from brine to cheese molecules occurs. Diffusion of water in cheese was mentioned by Katsiari *et al*. (2000, 2001).

By the end of the salting stage, cheese molecules reach a concentration of 2,25 g NaCl/100 g (i.e. 5.72% d.s.) rate which increases during the first days of the maturation process by 44% and reaching further a higher concentration of 3.35 g NaCl/100 g of cheese molecules (i.e. 8.25% d.s.). According to researches for the dry substance content of cheese, the diffusion stage is greatly decreased, the salt content being modified to a lesser degree, reaching after 35 days 3.55g/100 g of cheese molecules (i.e. 8.57% in d.s.), a steady rate.

The increase of the salt content along the process of cheese molecules maturation for 40 days is about 50% in comparison with the cheese molecules after salting stage. As for as the dry substance and salt content in brine cheeses are concerned the research is limited by the standards (Kaya, 2002).

3.3. Dynamic spoilage during the lactose stage

From the very beginning of the process producing brine cheese, the quality standard of lactose derived from the raw milk decreases (Shakeel-Ur-Rehman *et al*., 2004).

The milk lactose derived from the raw material is partially fermented by the glycolic activity of the lactic bacteria of the starter culture made use of; 90% of the milk lactose subject to coagulation is removed together with the whey (Rynne *et al*., 2007, McSweeney and Fox, 2004). The lactose content along the processing of brine cheese is shown in Table 2

Table 2.. *The lactose evolution content along the processing brine cheese*

The fabrication phase		**Lactose**		
		% cheese	**% D.S.**	**% comparison to the initial point**
after pressing		2,33	6,3	100
after salting		1,62	4,12	65,4
maturation, days	5	0,81	1,99	31,6
	10	0,33	0,79	12,5
	15	0,15	0,36	5,7
	20	0,01	0,024	0,4
	25	0	0	0
	30	0	0	0
	35	0	0	0
	40	0	0	0

Lactose degradation begins immediately after having added the starter culture and it keeps on during the process of coagulation and self pressing, that of salting and during the whole process of maturation. After the pressing stage, cheese contains a rate of 2.33% lactose/100g.

The salting process continuing at the same time with lactic fermentation, humidity content in cheese is

also reduced, fact which leads to a rate of decrease in the concentration of lactose from 6.3% d.s. to 4.12% d.s.

The intensive lactose diminution by 35% as compared to the initial concentration level along the salting stage is going on for the first five days of maturation (by 34 % as compared to the initial concentration level), then the process is slowering down for another following five days (by 20% as to the starting level of lactose), after which the level of spoilage seems pregnantly reduced by 7% in the first 10 to 15 days and by 5% for the next following five days.

After twenty days of maturation, cheese molecules contain lactose traces of a rate about 0.01% in cheese and of 0.024% in d.s. On the 25th day are fewer traces of lactose, the conclusion being that lactose fermentation in brine cheese can be traced on a higher level during the first five days, the concentration level decreasing by 32% as compared to the initial level.

3.4. The pH and acidity dynamics

The milk and the curdle acidity represents an important factor for the whey removal. The cheese acidity at a certain moment of the technological process is determined by the starting level of milk acidity and the lactic acid generated by the presence of the starter culture.

The cheese acidity level and the pH have a great importance as they influence the growth of microorganism and enzymatic activity along the maturation process, and also the rheological proprieties and the flavour (Watkinson *et al.*, 2001, Pappa *et al.*, 2007). The pH and acidity level during the process of cheese fabrication is shown in Table3.

Table 3. *The pH and acidity evolution along the brine cheese fabrication*

The fabrication phase		pH	Acidity		
			° T	Lactic acid	
				% cheese	% D.S.
after pressing		7,011	60,40	0,547	1,472
after salting		6,789	58,08	0,613	1,559
maturation, days	5	6,235	119,22	1,073	2,644
	10	5,801	132,56	1,193	2,885
	15	5,657	150,35	1,353	3,270
	20	5,412	153,57	1,382	3,338
	25	5,105	155,74	1,401	3,383
	30	5,007	155,74	1,402	3,384
	35	5,005	155,74	1,402	3,385
	40	5,002	155,75	1,402	3,385

The pH pot cheese levels after pressing (7.011) is decreasing while the salting process lowers by 0.2 units. The biggest pH diminution is registered during the first 10 days maturation, while during the next following fifteen days the diminution ranks slower by 0.7 units, after which the pH level remains steady.

pH evolution is related to lactose fermentation intensity and the acidity increase, but it is also influenced by buffer substances present in cheese. Cheese titrated acidity increases very quickly starting by the 5th day of maturation (60°T), and slowering down in the following ten days (20°T), after which it stays practically constant.

In the process of cheese maturation, the lactic acid level is twofold after the five days in comparison to that of the pressed fresh cheese (which rises to 2.644% d.s. as compared to 1.472% d.s.).

As it can be seen in table 3, after 25 days of maturation the content of lactic acid remains at the same level (3,383-3,385 % d.s.).

Calcium and phosphor inorganic subsalts are turned into soluble salts under the action of lactic acid.

The lactic acid and the lacteous formed during the maturation process of cheese are used by different bacteria resulting into new substances such as acetic acid and propionic acid, which plays a significant role in cheese taste and flavour.

Consequently, the lactic acid level resulted gradually decreases, thus favouring conditions for the future activity of lactic bacteria.

pH and acidity variation in brine cheese in different stages of fabrication are shown in Figure. 1

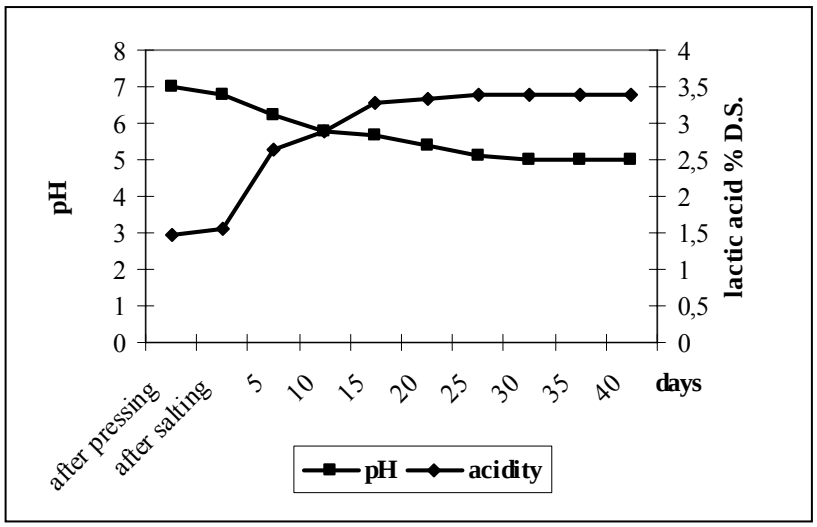


Figure 1. *The pH and acidity evolution in brine cheese*

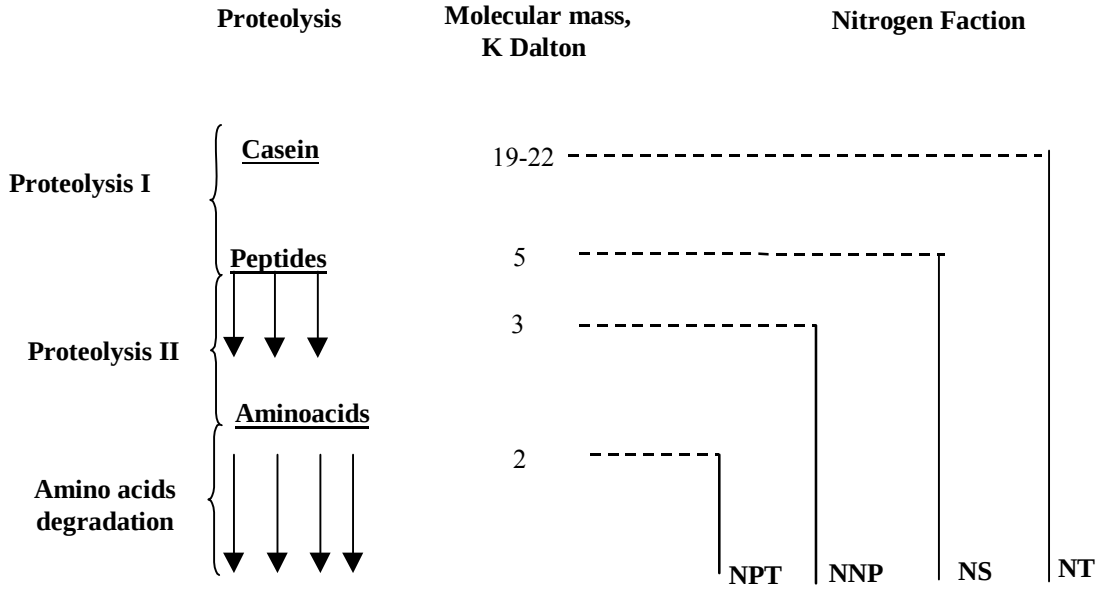
3.5. The nitrogen faction dynamics

The process of maturation displays gradually casein degradation. The research of proteolyses was studied considering the evolution of the nitrogen substances in brine cheese (Michaelidou *at al*., 2005, Mallatou *et al*., 2003, Michaelidou *et al*. 2003). Fraction divided nitrogen dosage in maturated cheese is shown in Figure 2.

At certain periods of time during the research, the following compounds were determined: total nitrogen (TN), water soluble nitrogen (WSN), soluble nitrogen at pH 4,6 (SN), soluble nitrogen in fosfotungstic acid (PTASN), soluble nitrogen in tricolour acetic acid 12% (TCASN), amine nitrogen (AN) and ammonia nitrogen (AmN); other nitrogen factions resulted from the proteolysis were also counted: protein nitrogen (PN=TN–TCASN), casein nitrogen (CN=TN–SN), peptide nitrogen – Large peptides (NP_dM=NSA–TCASN), peptide nitrogen–little peptides (NP_dm=TCASN–PTASN) so that by the end of the research the rate in cheese was 0.05%.

The values of these nitrogen factions are shown in Table 4 and Table 5. The little peptides content level of change bears a 1.5 times higher increase during the first 15 days, as compared to that of pressed cheese while during the next 10 days the same level goes to a rate of 3.5 times higher.

It was noticed, thereafter, a slower increase again in the 30th day after which the content level remains constant. Figure 3 and Figure 4 display the evolution of large and small peptides as related to TN and PN.



where: **NT**–total nitrogen; **NS**– soluble nitrogen at pH 4,6 in buffer acetate medium; **NNP**–no protein nitrogen (soluble in TCA 12%); **NPT**– fosfotungstic nitrogen (soluble in fosfotungstic acid 5%).

Figure 2. *The fractionated nitrogen dosage from maturated cheese*

Table 4. *Nitrogen factions determined for the brine cheese*

Fabrication phase		Nitrogen fractions											
		TN		WSN		TCASN		PTASN		AN		AmN	
		%	% D.S.	%	% D.S.	%	% D.S.	%	% D.S.	%	% D.S.	%	% D.S.
after pressing		3,031	8,200	0,165	0,44	0,091	0,24	0,016	0,04	0,015	0,04	0,005	0,012
after salting		3,224	8,197	0,208	0,53	0,129	0,33	0,027	0,07	0,022	0,05	0,009	0,023
maturation, days	5	3,328	8,199	0,247	0,61	0,166	0,41	0,060	0,14	0,070	0,17	0,011	0,030
	10	3,391	8,200	0,268	0,65	0,186	0,45	0,077	0,18	0,108	0,26	0,013	0,032
	15	3,392	8,199	0,282	0,68	0,203	0,49	0,092	0,22	0,132	0,32	0,015	0,036
	20	3,394	8,200	0,361	0,87	0,327	0,79	0,102	0,25	0,222	0,53	0,017	0,041
	25	3,395	8,200	0,555	1,34	0,496	1,2	0,109	0,26	0,347	0,84	0,020	0,048
	30	3,393	8,198	0,568	1,37	0,534	1,28	0,109	0,26	0,348	0,84	0,021	0,050
	35	3,393	8,199	0,581	1,4	0,547	1,32	0,111	0,27	0,349	0,84	0,022	0,053
	40	3,392	8,199	0,585	1,41	0,553	1,33	0,112	0,27	0,351	0,85	0,023	0,055

Table 5. *Nitrogen factions calculated for the brine cheese*

Fabrication phase		**Nitrogen fractions**							
		PN		**CN**		**Large peptides**		**Small peptides**	
		%	% D.S.	%	% D.S.	%	% D.S.	%	% D.S.
after pressing		2,940	7,95	2,866	7,75	0,074	0,20	0,075	0,20
after salting		3,095	7,87	3,019	7,67	0,079	0,20	0,102	0,25
maturation, days	5	3,162	7,79	3,082	7,59	0,081	0,20	0,106	0,26
	10	3,205	7,75	3,124	7,55	0,082	0,20	0,109	0,26
	15	3,188	7,70	3,111	7,52	0,079	0,19	0,111	0,27
	20	3,067	7,41	3,034	7,33	0,032	0,07	0,235	0,54
	25	2,899	7,00	2,868	6,92	0,032	0,07	0,387	0,94
	30	2,859	6,90	3,829	6,83	0,034	0,08	0,425	1,03
	35	2,846	6,87	2,816	6,80	0,032	0,07	0,442	1,06
	40	3,839	6,85	2,809	6,78	0,023	0,05	0,445	1,07

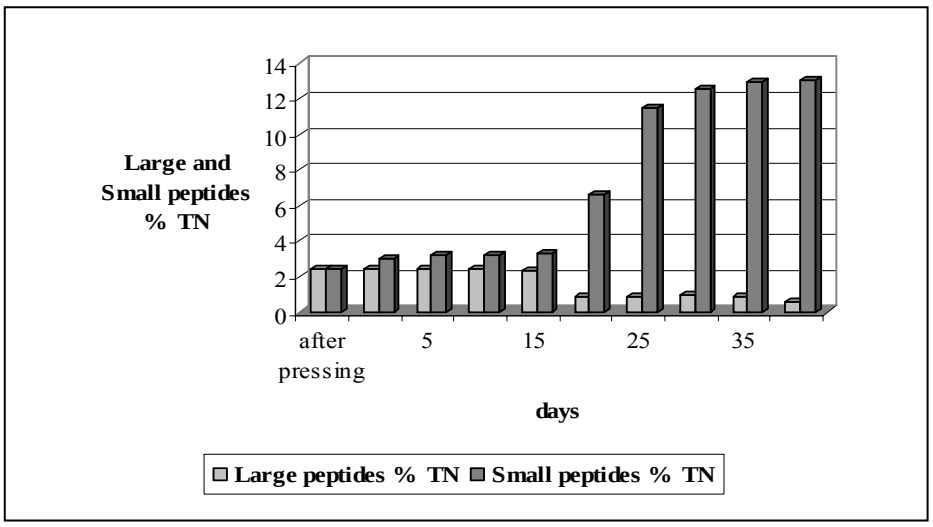


Figure 3. *Large and Small peptides evolution related to TN*

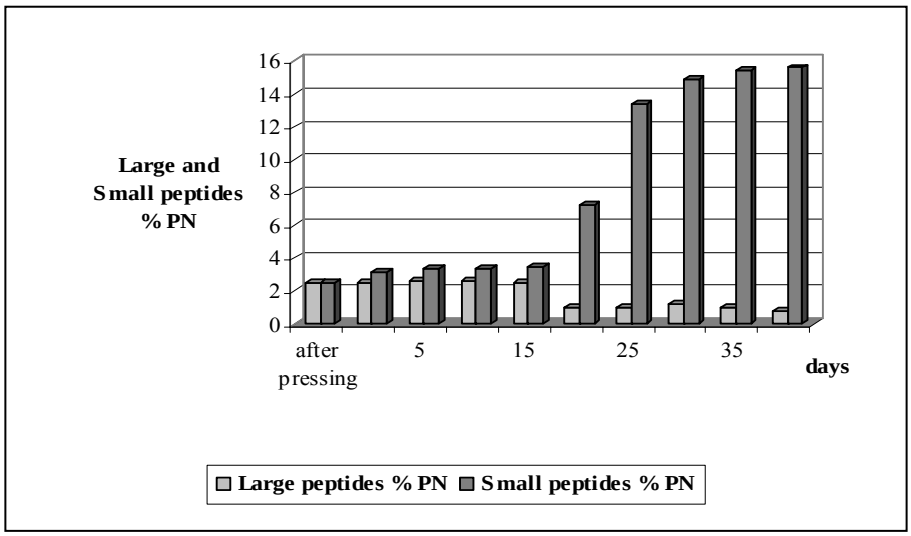


Figure 4. *Large and Small peptides evolution related to PN*

The final proteolysis product obtained from the amino acids desamination and ammonia nitrogen (NAc), is progressively accumulated in cheese during the 30 days and reaching levels of 4.2 times higher than in pressed cheese. During the following ten days the level of ammonia content is reduced

3.6. The monocalcium paracaseinate content evolution

The obtained curdle from the clot activity–dicalcium paracaseinate as gradually turned over the lactic acid into monocalcium paracaseinate soluble in a 5% NaCl solution at a temperature of 50-55 °C. That protein faction in the presence of salt plays the part of glue better than others paracaseinats and as a consequence it might influence, to a great degree the rheological cheese proprieties (Costin and Rotaru, 1994).

It is important to establish therefore, the rate of that compound during different stages of the process of producing cheese. The level of monocalcium paracaseinate after the salting is four times higher there that of the pressed pot cheese. The increase is higher in the first five days of the process of maturation by about 10 times than the level of pressed pot cheese as a consequence of an intense acidification process taking place during the same period of time.

The rate of increase of monocalcium paracaseinate quantity is lower after 15 days of maturation. A rate of 4.071% d.s. content, representing 50 % of the whole quantity of nitrogen, was found in brine cheese after 25 days of maturation. It is interesting that after 40 days of maturation, 67% of the total water insoluble factions is represented by the monocalcium paracaseinate nitrogen. In Table 6 is shown the monocalcium paracaseinate content during the maturation process.

Table 6. *The monocalcium paracaseinate variation content*

The fabrication phase		**Nitrogen from monocalcium paracaseinate**		**Monocalcium paracaseinate**	
		% D.S.	**% in comparison to the pressed curdle**	**% total nitrogen**	**% water insoluble nitrogen**
after pressing		0,074	100	0,90	0,95
after salting		0,295	398,6	3,60	3,85
maturation, days	5	0,739	998,6	9,01	9,74
	10	1,408	1902	17,17	18,65
	15	3,171	4285,1	38,67	72,17
	20	3,635	4912,1	44,32	49,60
	25	4,071	5501,3	49,65	59,34
	30	4,392	5935,1	53,57	64,40
	35	4,481	6055	54,65	65,90
	40	4,552	6155,6	55,52	67,05

4. Conclusions

The research's objective was the ripening evaluation process of the lactose fermentation dynamics and the degradation of the nitrogen factions.

After pressing, the dry substance content is increasing much faster along the salting process and in the first 15 maturation days, pursuant to a double diffusion process generated by the difference of the osmotic pressure between the brine and the liquid cheese phase. The excluding water process is

considerable decreasing, and after 30 days the process is stopped.

➔ The NaCl concentration from the brine cheese is increasing during the maturation time, especially in the first 5 days (1.5 times higher), subsequent the increasing rate is diminished

➔ The lactose substance is much fast fermented in the salting stage and in the first 5 maturation days (about 68% in comparison to the value of the pressed pot cheese).

➔ The lactose substance is also fast fermented during the salting process and in the first 5 maturation days (about 68% in comparison to the pot cheese level). After 15 days there remains at least a rate of 6% as compared to the initial lactose level while the process of fermentation draws towards and end after another 20 days of the maturation process.

➔ pH evolution is related to lactose fermentation intensity and to acidity increase, but it also bears the stamp of the buffer substances in the cheese product. During the first days of the maturation process and during the salting stage, pH rate of decreasing takes place faster (by 1.2 units) before it becomes slower and gets steady.

The similar dynamics governs the situation of titrating cheese acidity. After the first five days of the process of maturation the rate of acidity in lactic acid % D.S. is 1.7 times higher that in the salted soft cheese.

➔ The monocalcium paracaseinate making up, important for the rheological proprieties of cheese undergoes an intense process in the first days of maturation, concurrent with the accumulation of the lactic acid. After 15 days the increasing paracaseinate content is delayed by the decrease of the speed of the lactic acid obtaining process and the drive of the monocalcium paracaseinate proteolysis. After 30 maturation days 65% of the water insoluble nitrogen factions are represented by the monocalcium paracaseinate nitrogen, which can explain the characteristic structure of the brine cheese, as almost a friable paste, in comparison to the other maturated cheese types.

The dynamic factions: the water soluble nitrogen, protein nitrogen, the casein nitrogen, peptide nitrogen, amine nitrogen and ammonia nitrogen is obviously a higher rate of proteolysis in the 15 – 25 days period, succeeded by a shoot cut starting on the 30th day, after that, due to the low storing cheese temperature (under 10 °C), the proteolysis takes place very slowly.

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