Study of the Probiotic Telemea Cheese Maturation

Mădălina ULIESCU*, Gabriela ROTARU, Dănuț MOCANU**, Violeta STANCIU****

**Edmond Nicolau* High School Brăila, e-mail: madalina_uliescu@yahoo.com
**Bioingineering Dept., Faculty of Food Science and Engineering, University *Dunarea de Jos* of Galati,
47 Domneasca St., 800008, Tel./Fax: +40 236 460165, e-mail: Gabriela.Rotaru@ugal.ro

Abstract
The study refers to probiotic Telemea cheese making. To be considered probiotic product, Telemea cheese must contents minimum $1x10^6$ cfu / ml. For this reason is very important to determine probiotic bacteria viability in conditions of salts high concentrations and persistence in product after minimum 21 days of the brine maturation and during period at the freezing temperature. Researches are concerning with the comparison between probiotic Telemea cheese and classic Telemea cheese maturation dynamics.

Key words: probiotic cheese, proteolysis, lactose, nitrogenous fractions, cheese chemical analysis

Rezumat
Studiul a vizat realizarea de brânză telemea cu probiotice. Pentru a fi considerată produs probiotic brânza telemea trebuie să conțină minim $1 \cdot 10^6$ ufc/ml, drept pentru care este foarte important să se cerceteze viabilitatea bacteriilor probiotice la concentrații mari de sare şi persistența în produs după minim 21 de zile de la maturarea în saramură şi în timpul păstrării la temperatura de refrigerare. Cercetările au vizat urmărirea dinamicii maturării brânzei telemea probiotice comparativ cu brânza telemea clasică

Cuvinte cheie: brânză probiotică, proteoliză, lactoză, fracțiuni azotoase, analiza chimică a brînzeturilor

1. Introduction

Due to their chemical composition and high assimilation grade milk and dairy products, occupy a significant position in man rational diet, being one of the more accessible sources of the animal origin protein. Cheese represents an excellent food because of the nutritive high value, high bioavailability and consumption its good taste.

Nutritional interest for this food results, generally, is associated with the presence in its composition, in relative great ratio, of the great biological value protein, calcium, phosphorus and some vitamins, especially A and D. From all varieties of cheeses manufactured in our country, brine cheeses are most widespread, searched and appreciated by customers.

Telemea cheese and cascaval take the lead in consumption and represents approximately 60% from all cheeses made in Romania.

Cheese quality depends on many varieties of factors: raw milk composition, technological process parameters, bacteria types and storage, transportation and delivery conditions. Cheese as result of applied biotechnology, is one of the most complex and dynamic foods. In cheese making practice the utilization of started cultures are very important.

They contribute at obtaining sensorial characteristics and specific flavour. Each piece can be considered as a bio reactor in which many and complexes reactions are produced therefore the result is a final product with specifics sensorial characteristics. The complexity of the biotechnological processes in cheese making practice is characterized by many physical, chemical, biochemical and microbiological transformations.

Probiotic microorganisms are normal and important component of the intestinal micro-flora. If these are

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supplyed with foods in active form and in enough number, determine benefits effect on the health.

Probiotic products are getting nowdays and better place in studies of the specialists in food industry. There are probiotic fermented dairy products on the market. In concerning with the cheese importance in consumption, researches upon probiotic cheese making are very useful.

The study is focused on the probiotic Telemea cheese making, considering important role of the Telemea cheese in total consumption of all cheese from our country. Telemea cheese is a variety of the cheese with high content of salt (2,5–4%). To be considered probiotic product, Telemea cheese must content minimum 1×10^6 cfu/ml. So it is very important to determine probiotic bacteria viability at high salt concentrations and survival rate in product after minimum 21 days of the brine ripening in storage conditions at the freezing temperature. Researches are concerning with pursue probiotic Telemea cheese maturation dynamic comparing with clasic Telemea cheese.

2. Materials and methods

Two experimental varieties of Telemea cheese: with classical and probiotic culture were obtained. Cow milk from Brăila Stăncuța county with following characteristics: acidity 19ºT, density 1,028 kg/m^3, *p*H 6,6; fat 3,6% was used.

Milk was coagulated with Fromase–Chr.Hansen 22000TL from *Rhizomucor miehei* with coagulation power P_c = 1:150.000. For the classic Telemea o DVS starter culture–Danisco EZAL France CHOOZITMT1LYO10DC1 was used. This starter consists of: *Lactococcus lactis*, *Lactococcus cremoris*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. For the probiotic Telemea sample was used a mixture 1:1 DVS Danisco-EZAAL-France and YOMIX 205LYO250DCU cultures. The YOMIX 205LYO250DCU culture content was: *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium lactis* and *Lactobacillus acidophilus*.

Using classic process makes those both cheese types. At different cheese making process stage were analyzed: dry substance, acidity, *p*H, lactose, The proteolysis that occurs during ripening has been monitored from nitrogenous fraction analyses with fractionated precipitation and the extract obtained, by Kjeldahl methods.

The values obtained from experimental analysis are: ***TN***–total nitrogen, ***SN***–soluble nitrogen at pH 4,4 in buffer solution (citrate/hydrochloric acid), ***NPN***–non proteic nitrogen (soluble in 12% trichlor acetic acid), ***WSN***–water soluble nitrogen, ***PTSN***–phosphotungstic nitrogen (soluble in 5% phospho tungstic acid), ***AN***–amino nitrogen.

The values calculated from those are: ***PN*** – protein nitrogen=TN–NPN, ***CN*** – casein nitrogen = TN – SN, ***large peptides nitrogen*** = WSN – NPN, ***small peptides nitrogen*** = NPN – PTSN.

3. Results and discussion

In tables 1 and 2 it is shown the evolution of some cheese components from pressing to 40 days of maturation. Values of the dry matter content (figure 1) for both cheese types are almost the same. Thus, probiotic Telemea cheese has, after pressing, a smaller dry matter content (this cheese is softer) and during salting, the green cheese loses more whey and the dry matter content is now same as classic Telemea cheese.

Will have taken place during salting and the first 5 days of ripening, a high growth rate of the NaCl content (figure 3) until 3,35 g/100 g in classic cheese, then it is maintain almost constant (figure 2). The salt diffusion in probiotic Telemea cheese has taken place more slowly during in brine ripening until 2,64 g/100 g cheese after 5 days, then the growth is slowed down until 3,55 g/100 g cheese after 20 days, then it remained constant (figure 2b).

Lactose is the main energy source for the bacteria in cheese and it is metabolized to lactic acid via pyruvic acid, that are both important precursors of aroma and flavour compounds. The lactose content (figure 3) decreases fast during salting in the first 5 days of ripening. It is observed a faster decrease of the lactose level reported on the dry matter for the probiotic cheese during salting, probably due the dry substance level increase as a consequence of high amount of whey going into the brine.

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Table 1. Classic Telemea cheese – Variation of the principal components

	Component								
	D.S. %	NaCl		Acidity			Lactose		*p*H
		%	% D.S.	ºT	g lactic acid	% D.S.	%	% D.S.	
After pressing	36.96	0	0	60.40	0.544	1.47	2.33	6.3	7.011
After salting	39.33	2.14	5.44	68.08	0.612	1.56	1.62	4.12	6.789
After 5 days	40.59	3.35	8.25	119.22	1.073	2.64	0.81	1.99	6.235
After 10 days	41.35	3.39	8.19	132.56	1.193	2.88	0.33	0.79	5.801
After 15 days	41.37	3.47	8.39	150.35	1.353	3.27	0.15	0.36	5.657
After 20 days	41.39	3.51	8.48	153.57	1.382	3.34	0.01	0.024	5.415
After 25 days	41.40	3.55	8.57	155.75	1.402	3.38	0	0	5.105
After 30 days	41.39	3.55	8.58	155.75	1.402	3.39	0	0	5.007
After 35 days	41.38	3.55	8.58	155.75	1.402	3.39	0	0	5.005
After 40 days	41.37	3.55	8.58	155.75	1.402	3.39	0	0	5.002

Table 2. Probiotic Telemea cheese – Variation of the principal components

	Component								
	D.S. %	NaCl		Acidity			Lactose		*p*H
		%	% D.S.	ºT	g lactic acid	% D.S.	%	% D.S.	
After pressing	28.44	0	0	73.23	0.659	2.31	2.3	8.09	6.855
After salting	38.19	2.25	5.89	104.29	0.939	2.46	1.58	4.14	6.356
After 5 days	39.77	2.64	6.64	118.05	1.062	2.67	0.79	1.99	5.887
After 10 days	40.25	2.97	7.38	125.22	1.127	2.8	0.33	0.82	5.765
After 15 days	41.75	3.27	7.83	149.87	1.349	3.23	0.13	0.31	5.633
After 20 days	43.11	3.55	8.23	155.74	1.402	3.25	0.03	0.069	5.375
After 25 days	44.53	3.53	7.93	155.7	1.401	3.15	0	0	5.107
After 30 days	44.54	3.52	7.90	155.7	1.401	3.15	0	0	5.003
After 35 days	44.55	3.52	7.90	155.7	1.401	3.14	0	0	5.003
After 40 days	44.56	3.51	7.88	155.7	1.401	3.14	0	0	5.003

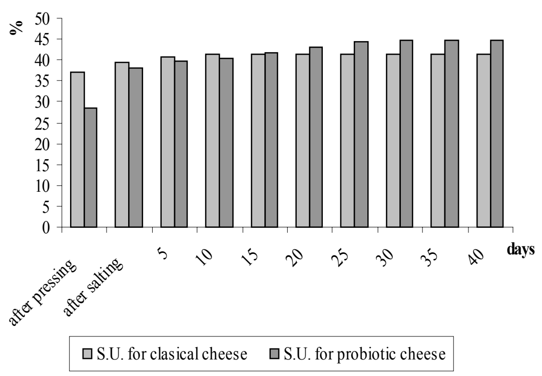

Figure 1. Dry matter content dynamic

After the first 10 days of ripening, the lactose level is reduced at 0,01 g/100 g in classic cheese and 0.03 g/100 g in probiotic cheese, the lactose being all fermented in 20–25 days period.

As a results of the intense lactose fermentation, the acidity level (figure 4) increases fast for the both cheese in the first 5 days, although an some lactic acid quantity reacts with dicalcium paracaseinate (DCP) and lactic acid loss take places. In the same time, a part of lactic acid goes with whey into brine.

During ripening, the acidity level decreases very slowly. The reported on dry matter high acidity values in probiotic cheese due high moisture of the cheese after pressing.

The *p*H values influences both the growth of microorganisms as well as the rate of biochemical reactions. The rate of acid production coupled with the loss of lactose determines the decrease of the *p*H value more intense in first stage – after pressing to 5 days. The variation is from 7.011 to 5.002 g/100 g in classic cheese and from 6.885 to 5.003 g/100 g in probiotic cheese. Then was a slowly evolution noticed the 25th day is related with the lactose fermentation. Until the experimental period ends in, 40 days, *p*H values remains almost unchanging.

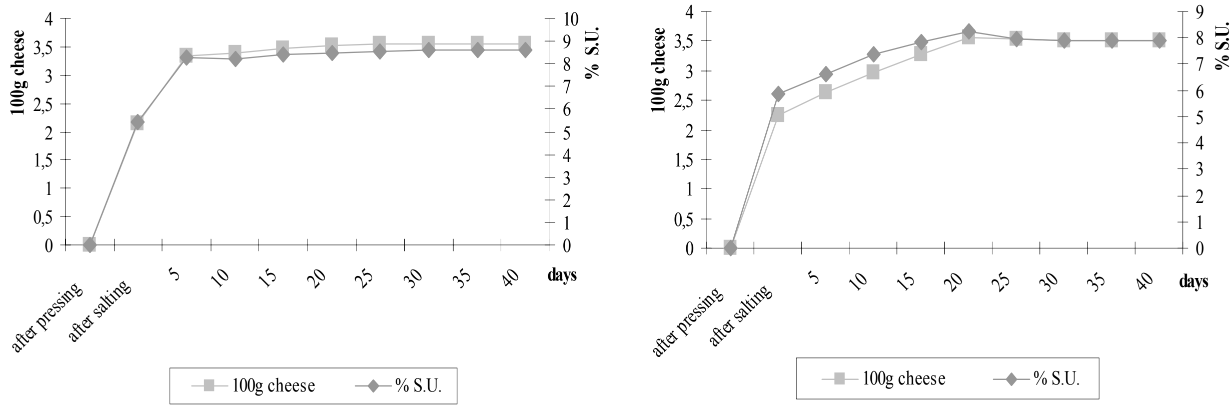

Figure 2. Sodiyum chlorid content variation for a) classic and b) probiotic Telemea cheese

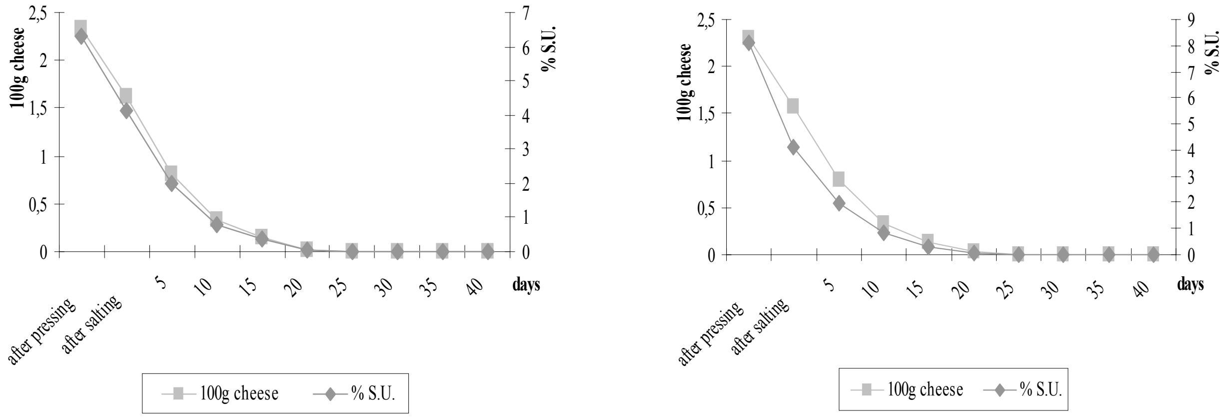

Figure 3. Lactose level variation for a) classic and b) probiotic Telemea cheese

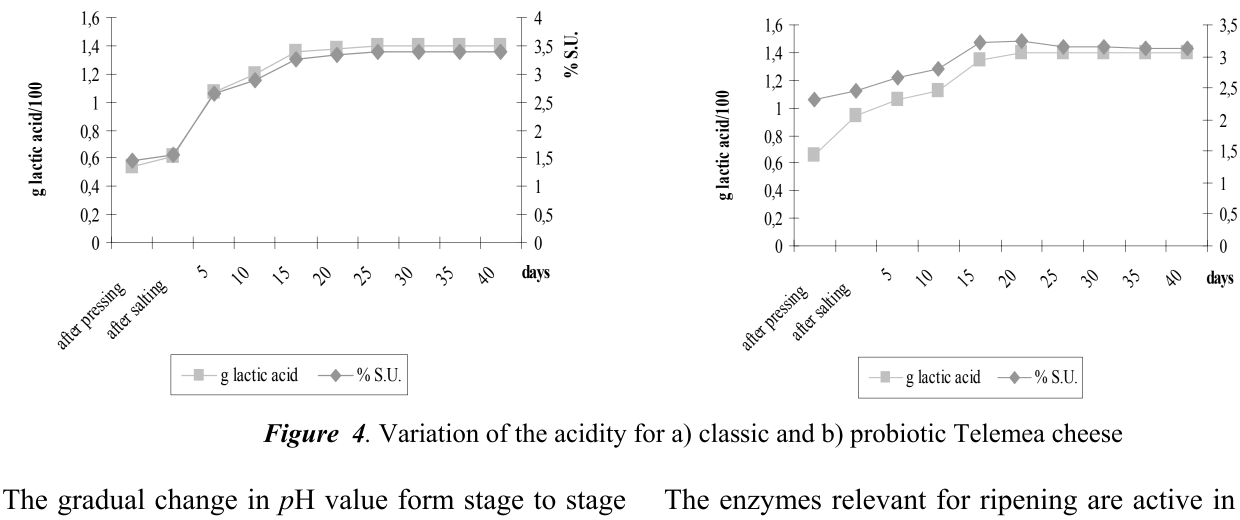

Figure 4. Variation of the acidity for a) classic and b) probiotic Telemea cheese

The gradual change in *p*H value form stage to stage enables ripening to proceed in a somewhat stepwise fashion so that, at any time, the nitrogen compounds reach a specific level in both cheese (tables 3 and 4).

The enzymes relevant for ripening are active in this pH region and these *p*H values tend to stimulate hydrolysis of fats and the production of nitrogen soluble substances, aminonitrogen and ammonia.

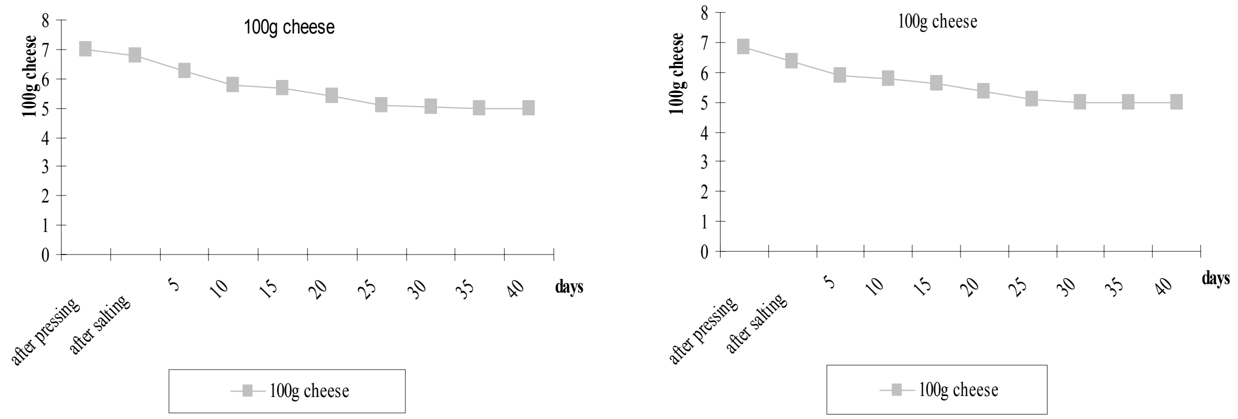

Figure 5. Variation of the *p*H for a) classic and b) probiotic Telemea cheese

Table 3. Nitrogenous fractions determinated for classic Telemea cheese

	Nitrogenous Fractions											
	TN		WSN		SN 4.4		TCASN		PTASN		AN	
	%	% D.S.	%	% D.S	%	% D.S	%	% D.S	%	% D.S	%	% D.S
After pressing	3.031	8.200	0.165	0.44	0.165	0.45	0.091	0.24	0.016	0.04	0.015	0.04
After salting	3.224	8.197	0.208	0.53	0.205	0.52	0.129	0.33	0.027	0.07	0.022	0.05
After 5 days	3.328	8.199	0.247	0.61	0.246	0.61	0.166	0.41	0.060	0.14	0.070	0.17
After 10 days	3.391	8.200	0.268	0.65	0.267	0.64	0.186	0.45	0.077	0.18	0.108	0.26
After 15 days	3.392	8.199	0.282	0.68	0.280	0.67	0.203	0.49	0.092	0.22	0.132	0.32
After 20 days	3.394	8.200	0.361	0.87	0.360	0.87	0.327	0.79	0.102	0.25	0.222	0.53
After 25 days	3.395	8.200	0.555	1.34	0.527	1.27	0.496	1.2	0.109	0.26	0.347	0.84
After 30 days	3.393	8.198	0.568	1.37	0.564	1.36	0.534	1.29	0.103	0.25	0.348	0.84
After 35 days	3.393	8.199	0.581	1.4	0.577	1.39	0.547	1.32	0.099	0.24	0.349	0.84
After 40 days	3.392	8.199	0.585	1.41	0.583	1.41	0.553	1.33	0.095	0.23	0.351	0.85

Table 4. Nitrogenous fractions determinated for probiotic Telemea cheese

	Nitrogenous Fractions											
	TN		WSN		SN 4.4		TCASN		PTASN		AN	
	%	% D.S.	%	% D.S	%	% D.S	%	% D.S	%	% D.S	%	% D.S
After pressing	2.329	8.189	0.078	0.27	0.073	0.26	0.020	0.07	0.008	0.03	0.008	0.03
After salting	3.128	8.190	0.113	0.29	0.106	0.28	0.034	0.09	0.019	0.05	0.011	0.03
After 5 days	3.257	8.189	0.141	0.35	0.134	0.34	0.060	0.15	0.032	0.08	0.024	0.06
After 10 days	3.298	8.193	0.227	0.56	0.220	0.55	0.145	0.36	0.072	0.18	0.068	0.17
After 15 days	3.419	8.189	0.273	0.63	0.266	0.64	0.192	0.46	0.104	0.25	0.083	0.20
After 20 days	3.531	8.191	0.350	0.81	0.343	0.79	0.271	0.62	0.143	0.33	0.116	0.27
After 25 days	3.647	8.190	0.547	1.23	0.522	1.17	0.468	1.05	0.193	0.43	0.171	0.38
After 30 days	3.648	8.190	0.560	1.26	0.561	1.26	0.496	1.11	0.200	0.45	0.173	0.39
After 35 days	3.648	8.189	0.573	1.29	0.597	1.34	0.524	1.17	0.203	0.45	0.175	0.39
After 40 days	3.649	8.189	0.585	1.31	0.633	1.42	0.552	1.23	0.207	0.46	0.179	0.40

The protein-nitrogen, casein-nitrogen, large peptides nitrogen and small peptides nitrogen resulted from proteolysis are calculated (tables 5 and 6). This nitrogen fractions serve to follow some ripening factor variations (tables 7 and 8). As most of the whey proteins are lost during manufacture, the

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dominant protein in cheese, casein is broken down by enzymes in proteases and polypeptidases from starters, peptides arising from the degradation of proteins exhibit flavours according to those amino acids that are terminal in the peptide chain. Proteolysis is probably the most important biochemical event during ripening of cheese.

Ripening index (figure 6) as % WSN/TN increases in the first 25 days. After 25 days maturation index increases slowly due to maintaining of the cheese under 10ºC. During all experimental period the primary proteolysis of the probiotic cheese is slower than in classic cheese. Secondary proteolysis products include peptides and amino acids that are soluble in the aqueous phase of cheese and extractable with water. The WSN fraction contains whey proteins, proteose–peptone, low molecular weight peptides derived from casein hydrolysis and free amino acids.

This fraction increased rapidly from the first day to d 25, followed by a slower increase at the end of the maturation period.

Rennet is the main proteolytic agent responsible for the production of large peptides from casein, whereas bacterial enzymes cause the formation of short-chain peptides, amino acids, ammonia and other minor compounds that are soluble in 12 % TCA The formation of TCASN followed a pattern similar to WSN evolution i.e. a large increase the first day to d 25 and a light increase thereafter. The PTASN is an estimation of the contents of amino acids and very small peptides. Increases of the PTASN were more linear and regular throughout ripening.

Table 5. Nitrogenous fractions calculated for classic Telemea cheese

	Nitrogenous Fractions											
	PN		CN *		CN **		Large peptides *		Large peptides **		Small peptides	
	%	% D.S.	%	% D.S	%	% D.S	%	% D.S	%	% D.S	%	% D.S
After pressing	2.94	7.96	2.866	7.76	2.866	7.75	0.074	0.2	0.074	0.21	0.075	0.2
After salting	3.095	7.867	3.016	7.667	3.019	7.677	0.079	0.2	0.076	0.19	0.102	0.26
After 5 days	3.162	7.789	3.081	7.589	3.082	7.589	0.081	0.2	0.08	0.2	0.106	0.27
After 10 days	3.205	7.75	3.123	7.55	3.124	7.56	0.082	0.2	0.081	0.19	0.109	0.27
After 15 days	3.189	7.709	3.11	7.519	3.112	7.529	0.079	0.19	0.077	0.18	0.111	0.27
After 20 days	3.067	7.41	3.03	7.33	3.034	7.33	0.032	0.07	0.033	0.08	0.225	0.54
After 25 days	2.889	7	2.84	6.86	2.868	6.93	0.030	0.07	0.031	0.07	0.387	0.94
After 30 days	2.859	6.908	2.825	6.828	2.829	6.838	0.030	0.07	0.03	0.07	0.431	1.04
After 35 days	2.846	6.879	2.812	6.799	2.816	6.809	0.030	0.07	0.03	0.07	0.448	1.08
After 40 days	2.809	6.789	2.807	6.789	2.809	6.789	0.023	0.05	0.03	0.08	0.458	1.1

Table 6. Nitrogenous fractions calculated for probiotic Telemea cheese

	Nitrogenous Fractions											
	PN		CN *		CN **		Large peptides *		Large peptides **		Small peptides	
	%	% D.S.	%	% D.S	%	% D.S	%	% D.S	%	% D.S	%	% D.S
After pressing	2.309	8.119	2.251	7.919	2.256	7.929	0.058	0.2	0.053	0.19	0.012	0.04
After salting	3.094	8.1	3.015	7.9	3.022	7.91	0.079	0.2	0.072	0.19	0.015	0.04
After 5 days	3.197	8.039	3.116	7.839	3.123	7.849	0.081	0.2	0.074	0.19	0.028	0.07
After 10 days	3.153	7.833	3.071	7.633	3.078	7.643	0.082	0.2	0.075	0.19	0.072	0.18
After 15 days	3.227	7.729	3.146	7.559	3.153	7.549	0.081	0.17	0.074	0.18	0.088	0.21
After 20 days	3.26	7.571	3.181	7.381	3.188	7.401	0.079	0.19	0.072	0.17	0.128	0.29
After 25 days	3.179	7.14	3.1	6.96	3.125	7.02	0.079	0.18	0.054	0.12	0.275	0.62
After 30 days	3.152	7.18	3.088	6.93	3.087	6.93	0.064	0.15	0.065	0.15	0.269	0.66
After 35 days	3.124	7.019	3.075	6.899	3.051	6.849	0.049	0.12	0.073	0.17	0.321	0.72
After 40 days	3.097	6.959	3.064	6.879	3.016	6.769	0.033	0.08	0.081	0.19	0.345	0.77

*from water plan; ** from citrat *p*H 4.4 plan.

The NH_2–N content which is highly sensitive to free amino acids, increased regularly throughout ripening and showed a good correlation with TCASN and PTASN. Cheese age was the main significant factor determining the level of all N fractions.

The ripening depth (figure 7) measured at the end of proteolysis becomes significantly after 5 days.

In the first period occurs very intense protein hydrolysis resulting in large peptide formation. As well as ripening index, ripening depth is more reduced on probiotic cheese. In 20–25 days period both ripening index and ripening depth are very intense. The freezing and storage of the cheese under 10ºC stoped hydrolysis that could alter the taste and texture of the cheese. In the first 5 days period the two cheese types difference concerning casein hydrolysis is high. Casein in probiotic cheese hydrolyzed more slowly.

Table 7. Cheese maturation index a) Classic Telemea Cheese, b) Probiotic Telemea Cheese

	Maturation level, %		Maturation profoudness, %		Casein proteolysis index, %		Peptide proteolysis index, %		Amine proteolysis index, %	
	a	b	a	b	a	b	a	b	a	b
After pressing	5.444	3.349	0.66	0.472	0.946	0.966	0.0492	0.03	0.00495	0.00343
After salting	6.452	3.613	0.962	0.607	0.936	0.964	0.0561	0.03	0.00682	0.00352
After 5 days	7.422	4.329	2.43	1.105	0.926	0.957	0.0562	0.033	0.021	0.00737
After 10 days	7.903	6.883	3.57	2.668	0.921	0.931	0.0563	0.047	0.0318	0.0206
After 15 days	8.314	7.985	4.33	3.393	0.917	0.92	0.056	0.049	0.0389	0.0243
After 20 days	10.636	9.912	7.04	4.503	0.894	0.901	0.0757	0.059	0.0654	0.0329
After 25 days	16.348	14.999	10.8	7.349	0.845	0.85	0.123	0.097	0.102	0.0469
After 30 days	16.74	15.351	10.9	7.429	0.834	0.846	0.134	0.099	0.103	0.0474
After 35 days	17.123	15.707	10.9	7.511	0.83	0.843	0.139	0.101	0.103	0.048
After 40 days	17.246	16.032	11	7.701	0.828	0.84	0.141	0.104	0.103	0.0491

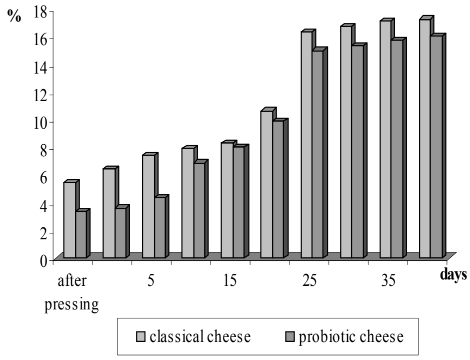

Figure 6. Ripening level variation

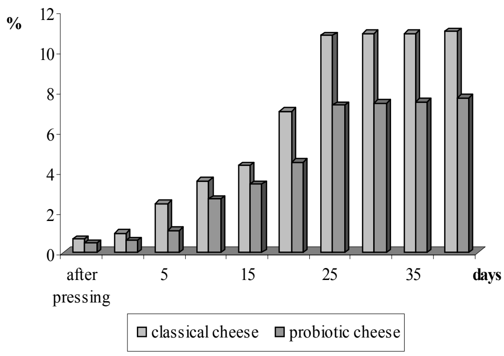

Figure 7. Ripening depth index variation

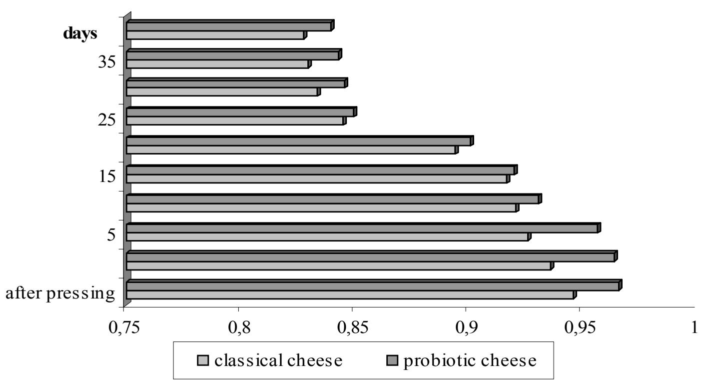

Figure 8. The casein proteolysis index

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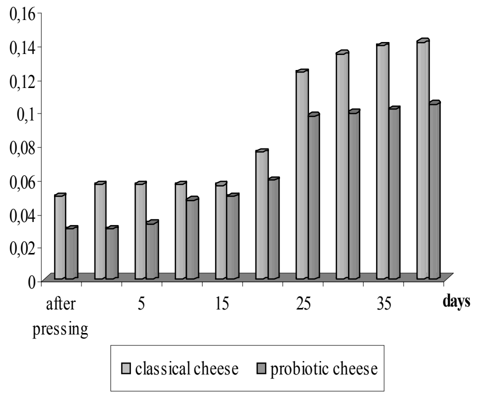

Figure 9. The peptide proteolysis index variation

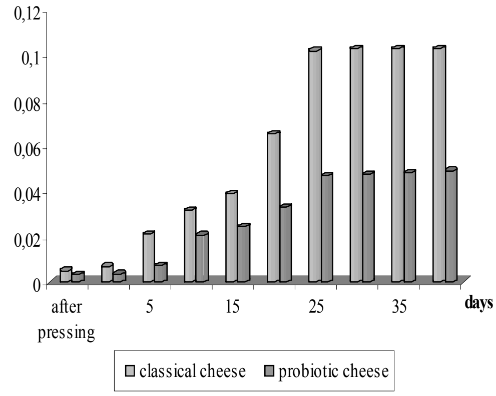

Figure 10. Amine proteolysis index variation

Hydrolysis products from casein are probably due to degradation of low molecular weight peptides and amino acids. In 10–20 days period the proteolysis occurs almost equally intense for both cheese. In 20–25 days period peptide proteolysis index is higher on classic cheese due to more intense proteolysis. In 20–25 day period the increased peptide index is higher for both cheese, than proteolysis occurry slowly due to low temperature of storage and the index value increases very little. A good correlation coefficient is between the total amount of peptides and WSN indicating that the evaluation of the peptide content can be used as a proteolytic index. In the first 5 days, proteolysis end products accumulation has reduced and in 5–25 days period it is pronounced, the index value remain constant. In probiotic cheese amines has ½ amount from classic cheese. Ripening depth is more reduced on probiotic cheese.

4. Conclusions

Lactose degradation in cheese ripening process has major involvement for all compounds transformation dynamics through influence on micro-flora development, enzyme activity, DCP obtained from enzyme coagulation and sensorial characteristics.

The *p*H has a high influence on cheese quality. If *p*H is not at necessary value, coliform bacteria and other contamination micro organisms which are fermenting lactose are able to multiply themselves and produce cheese defects as: excessive souring, hard friable pasta consistence, non uniform ripening, early gas production, reticular structure.

Following lactose intense fermentation, the acidity for both cheese increases fast, lactic acid reacts with dicalcium paracaseinate to form mono calcium paracaseinate which has property to melt and mix in salts solution, with of the curd grains to form an elastic, uniform, compact pasta.

At some acidity and in presence of few nutritive substance quantities (lactose and salts substances), bacteria begins to die and as a result the enzyme complex is freed. Enzymes break down casein splitting products from casein used coagulation enzyme and microorganism cells themselves.

During ripening, the acidity decreases very slowly. The Maturation demonstrate more slowly hydrolysis of casein in probiotic cheese.

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University *Dunarea de Jos* of Galati
Faculty of Food Science and Engineering
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