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Effect of total bacteria number and protein content of raw milk on probiotic bacteria multiplication

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ABSTRACT

Considering that the quality of raw milk is a prerequisite condition to obtain a good quality probiotic yoghurt, our studies aimed the measurement of milk factors which can affect the multiplication of probiotic lactic acid bacteria (LABs) Lactobacillus acidophilus (LA-5) and Bifidobacterium (BB-12) strains from Christian Hansen company (Danmark). We studied comparatively raw and pasteurized milk chemical composition and the correlations between the spontaneous microbial flora (expressed in CFU=colony formatting unit) found in milk samples and the impact of this flora on the multiplication of LABs. We investigated as well the effect milk proteins on pH and LAB development, the influence of NTG (number of total bacteria), on lactic fermentations and LA-5 (Lactobacillus acidophilus) and BB-12 activities. Generally the BB-12 (Bifidobacterium) activities were lower comparing with LA-5 and multiplication of both strains was reversely correlated with NTG values. Protein content of raw milk has minor influence on LA-5 and BB-12 multiplication , but it influences structure of probiotic yogurt.
(Keywords: yoghurt, probiotics, prebiotics, NTG, pasteurization)

ÖSSZEFOGLALÁS

A nyerstej összcsíraszámának és fehérjetartalmának hatása a probiotikus baktériumok szaporodására

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Figyelembe véve, hogy a nyerstej minősége közvetlen módon befolyásolja a probiotikus joghurt gyártását, tanulmányunk célja azon paraméterek vizsgálata volt, amelyek befolyásolhatják a probiotikus baktériumok szaporodását. (Lactobacillus acidophilus és Bifidobacterium BB-12). Összehasonlítottuk a fent említett baktériumok fejlődését nyers és pasztőrözött tejben, vizsgáltuk a nyerstej eredeti mikroflórájának összcsíraszámát, beltartalmi értekeinek hatását. Tanulmányoztuk a tejfehérje hatását a pH alakulására, az összcsíraszám hatását a tejsavas erjedésre, valamint az LA-5 és BB-12 baktériumok aktivitására. Kísérleteink alapján megállapítottuk, hogy a nyerstej összcsíraszáma fordítottan arányos a probiotikus baktériumok szaporodási sebességével. Az LA-5 baktérium ugyanazon körülmények között aktívabbnak bizonyult, mint a BB-12. A tejfehérje nem bizonyult döntő jelentőségűnek a probiotikus baktériumok szaporodásában, de fontos szerepet játszik a joghurt (végtermék) szerkezetének alakulásában.
(Kulcsszavak: joghurt, probiotikum , prebiotikum, csiraszám, pasztőrözés)

INTRODUCTION

Yoghurt is a long time known and appreciated dairy product, obtained traditionally by the spontaneous or induced lactic fermentation of milk. The microbiology of lactic-producing bacteria and the fermentation biochemistry and technology of yoghurt is well documented (*Apostu and Barzoi*, 2002; *Banu*, 2002; *Banu and Moraru*, 1972; *Costin*, 2005; *Socaciu*, 2001).

The term "probiotic" is known since 1903 when the benefic actions of *Lactobacillus acidophilus* strains were observed in human intestine, and the term of "prebiotic" is known since 1961, and define the substances, generally natural ingredients or microorganisms which improve the intestinal equilibrium and defense against pathological bacteria (*Brengmark and Martindale,* 2006; *Costin and Segal,* 2001; *Macrovei and Costin,* 2006; *Tomasik and Tomasik,* 2006).

Yoghurt, by its high content in lactic acid bacteria (LABs) possesses antimicrobial activity *in vitro* against a wide variety of Gram-positive and Gram-negative bacteria, as well as some fungi. The exact cause of inhibition is not fully known, but may be due to the antagonist action of LAB species which prevent the adherence, establishment, replication, and/or pathogenic action of certain enteropathogenes. To improve continuously the quality of yoghurts, preservation of probiotic characteristics and the shelf-life of live LABs, with improved capacity of fermentation, are needed (*Gropal,* 2007; *Kleebezen et al.,* 2006; *Shah,* 2007; *Reid,* 2003).

Among many strains, *Lactobacillus acidophilus* and *Bifidobacterium spp.* are the best candidates to be used, alone or in combinations as lactic fermenting microorganisms with high probiotic activity (*Kailaspathy and Rybka,* 1997). Important factor which influence the development and survival rate of probiotic LAB is the milk quality and its bacterial flora. It is known that the quality of raw milk in Romania is still an unsolved problem, since the number of total germs and of somatic cells found in milk is higher than the permitted level in European Union (Total Bacteria Number<100000 CFU/ml, Somatic Cell Count<400000CFU/ml) (*Banu,* 2002; *Banu and Moraru*, 1972).

Considering that the quality of raw milk is a prerequisite condition for obtaining a good quality probiotic yoghurt, our studies aimed the measurement of main milk factors which can affect the multiplication of both probiotic-forming bacteria *Lactobacillus acidophilus* (LA) and *Bifidobacterium* (BB-12). We studied comparatively the raw and pasteurized milk, the correlations between the spontaneous microbial flora found in milk samples and its impact on multiplication of probiotic bacteria.

MATERIAL AND METHOD

The samples of cow milk originated from the region of Bisericani and the tests were made at NIZO, Netherlands and at S.C. Gordon Prod Bisericani, at the company's authorized lab. For experiments we used two bacterial strains, *Lactobacillus acidophilus* and *Bifidobacterium BB-12* provided by Christian Hansen comp. The media used for the storage and determinations of bacterial multiplication were MRS agar for LA-5 the nutritive, sorbitol agar (Sanimed) used for the determination of total bacteria number from yogurts. To make measurements, the raw milk, after cooling, was inoculated with both bacterial strains at three dilutions (10^{-1}, 10^{-2} and 10^{-3}) and incubated for 72 hrs. The counting of bacteria was made after 48 and 72 hrs of incubation. For LA-5 and BB-12 the incubation was 38–43 °C. All samples were done in duplicate.

To determine the NTG we used bacterial counter IBC m Bactocount, produced by Bentley in USA, for determination of phosphatase activity a control test from Biokom

company, Bulgaria. The pH was determined by using the lab pH meter WTW315i, imported by Viola company (Romania), produced by WTW company, Germany, protein content of raw milk by Lactostar milk analyzer produced by Gerber company (Germany). Density of raw milk was determined by lacto-densimeter on 20 °C (density of milk may be read directly on densimeter's scale).

RESULTS AND DISCUSSION

The composition of milk samples (raw and pasteurized) before to be inoculated with probiotic strains

The main characteristics of raw milk comparing with the pasteurized milk are presented in *Table 1.* The fat, protein, lactose content of milk are measured in percent, the microorganisms number in CFU (colony formating unit). By these mesurements we studied the effect of pasteurizing process on raw milk composition and properties.

Table 1.

Composition and properties of raw and pasteurized milk samples

Milk samples (1)	Total protein (4) g/100 g	Lactose (5) g/100 g	Fat (6) g/100 g	Density (7) kg/l	Aerobic psyhotrophic microorganisms (8) (CFU/ml)	Phosphatase activity (9)
Raw milk (2)	3.28	4.41	3.80	1.0291	$1.4 \cdot 10^7$	positive
Pasteurized milk (3)	3.27	4.53	3.77	1.0287	$9.6 \cdot 10^4$	negative

1. táblázat: A nyers- és pasztőrözött tejminták kémiai paraméterei

Tejminta(1), Nyerstej(2), Pasztőrözött tej(3), Fehérje(4), Tejcukor(5), Zsír(6), Sűrűség(7), Aerob psyhotrophic mikroorganizmus (8), Foszfatáz aktivítás(9)

There were no significant differences between density, proteins, lactose and fat contents in raw vs pasteurized milk, but a significant decrease of aerobic psyhotropic microorganims. Phoshatase activity was measured gfrom raw and pasteurized milk, also, because we used a rapid test, and in this way we could check its activity.

Relations between the NTG found in raw and pasteurized milk and their effects on the multiplication probiotic strains of LA-5 and BB-12. We observed that the initial milk NTG influenced significantly the probiotic bacteria evolution (*Table 2*). We found out that pasteurization at 72 °C was not enough efficient and even after 95 °C pasteurization, the presence of microorganisms can affect the probiotic development.

Determination of total bacteria number in milk samples

Number of total bacteria (NTG) was determined by IBCm Bactocount, and the results are presented in *Figure 1* on the left are presented the frequency of bacteria, on right a report of bacteria intensity in milk sample. Result of total bacteria number will appear automatically on sceen in function of frequency and intensity. IBCm Bactocount milk analyzer is calculating total bacteria number on base of these parameters.

To determine the influence of total bacteria number on LA-5 and BB-12 multiplication it was important selecting of raw milks with different total bacteria number. It was important, also, that chemical properties of milk (fat content, protein content, lactose content) to be similar. For inoculation we used pure probiotic strains BB-12 and LA-5 in a dosage: 0.025 units g/liter. Influence of total bacteria number on LA-5 and BB-12 multiplication was measured off by determining of acidity, pH of final product.

Raw milk's NTG influences directly in negativ way total bacteria number of pasteurized milk. Acidity , pH, lactic acid content of probiotic yoghurt obtained from raw milk with high NTG will be lower. Samples with NTG>500000 CFU/ml after pasteurizing process will have a total bacteria number>6000 CFU/ml, and this concurrent microflora will affect the multiplication of LA-5 and BB-12 probiotic strains.

BB-12 bacteria is more sensitive than LA-5. In same conditions, after 6 hours of incubation the acidity and lactic acid content of yoghurt was lower with BB-12. Even LA-5 is more resistant bacteria, sensorial propertes of yogurt with BB-12 were better (taste, smell, structure). So for obtaining probiotic yogurt NTG of raw milk has major influence. A high bacteria number produces a concurrent culture is inhibiting LA-5 or BB-12 bacteria multiplication and; quality and safety of final product is affected.

Table 2.

The influence of NTG raw and pasteurized milk NTG on multiplication of LA-5 and BB-12, at concentrations of 0.025 g/l, temperature of milk: 38 ºC, 6 hours

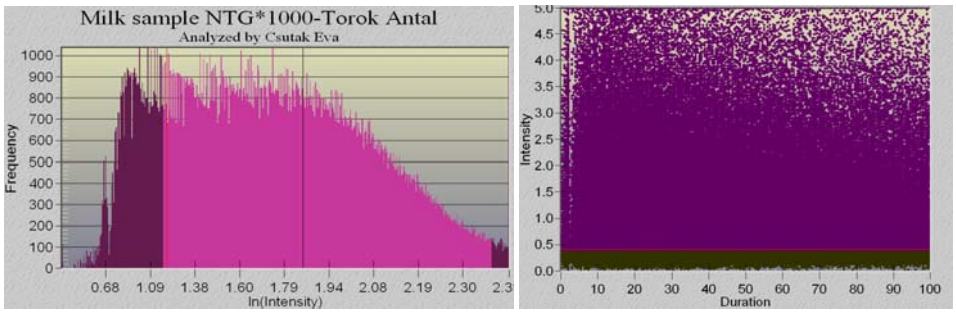
Sample (1)	NTG of milk saple by IBCm BactoCount (2) CFU/ml*1000000	NTG of pasteurized milk (3) CFU/ml*1000	Titrable acidity (4) LA-5, Th°*10	pH, LA-5,	Titrable acidity BB-12, Th°*10	pH, BB-12,
1	10.62	10.2	7.1	4.79	7.0	4.74
2	8.58	9.45	7	4.78	7	4.76
3	1.70	7.6	8.1	4.65	8.0	4.62
4	1.59	8.1	7.8	4.68	7.6	4.67
5	0.99	6.35	8.2	4.59	8.1	4.53
6	0.36	6.4	8.4	4.56	8.1	4.54
7	0.27	6.1	7.6	4.67	7.2	4.62
8	0.11	5.15	8.4	4.61	8.4	4.60
9	0.09	4.85	8.8	4.78	8.6	4.72

3. táblázat: A nyers és pasztőrözött tej csiraszámának hatása az LA-5 es BB-12 baktériumok szaporodására (0,025 g/l kultúra koncentráció, 38 °C inkubálási hőmérséklet, 6 óra)

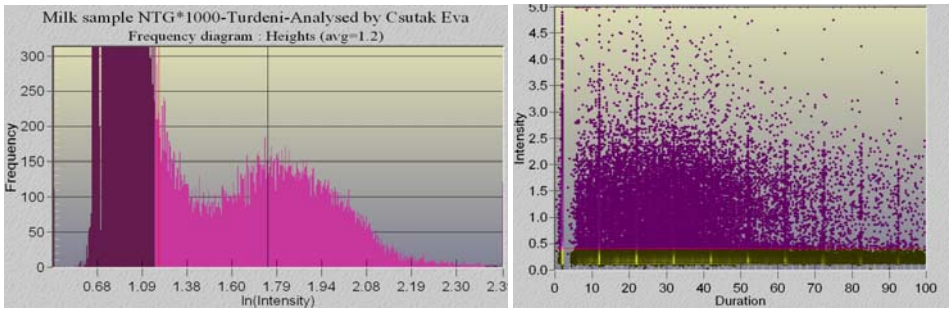
Mintaszám(1), Tejminta összbaktérium száma (IBCm Bactocount)(2), Pasztörőzött tej összbaktérium száma(3), Titrálható savasság(4)

Figure 1.

Determination of total bacteria number in milk samples



NTG: 10621099CFU/ml-sample 1.



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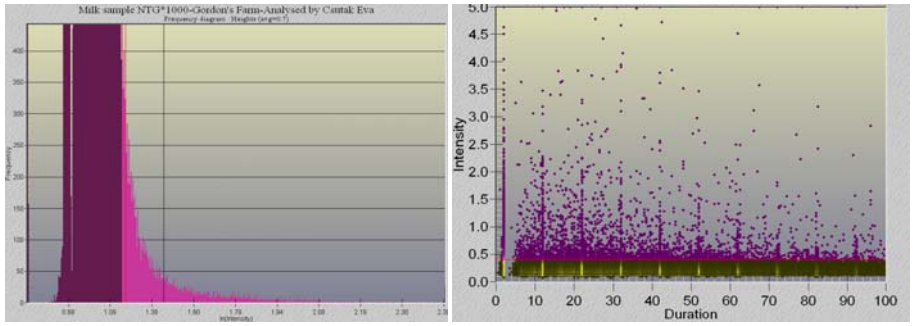
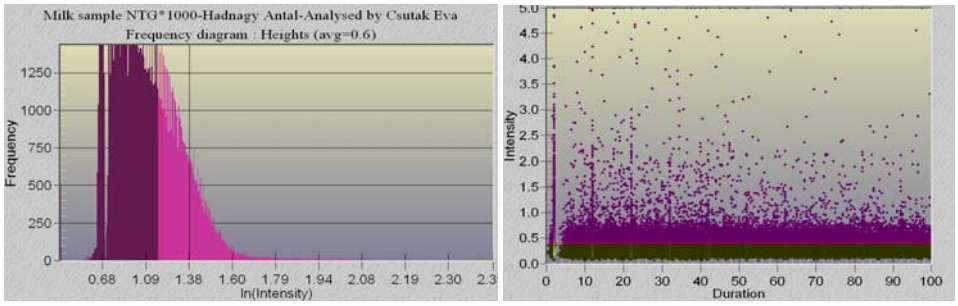


NTG: 357856CFU/ml-sample 4



NTG: 1702388CFU/ml-sample 2

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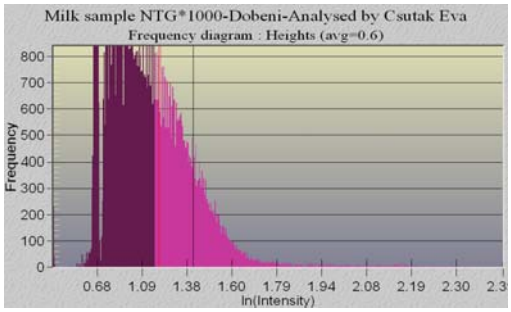

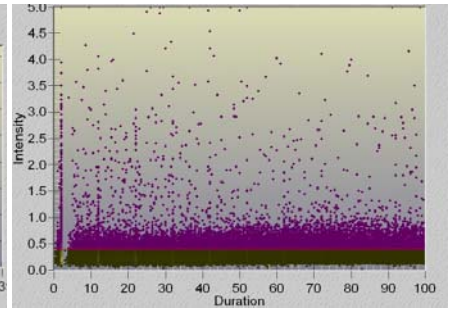

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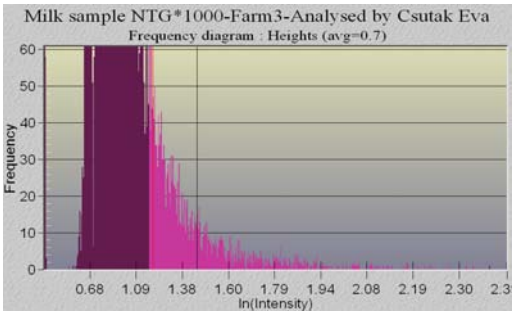
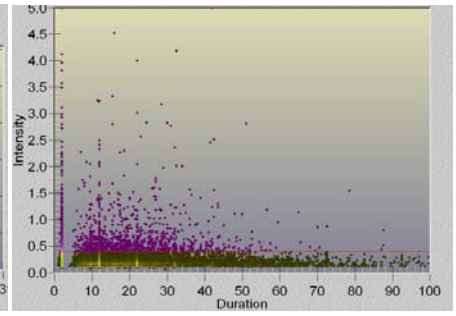
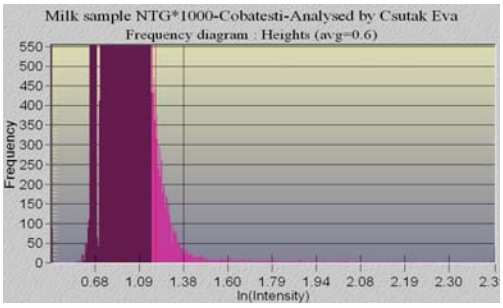
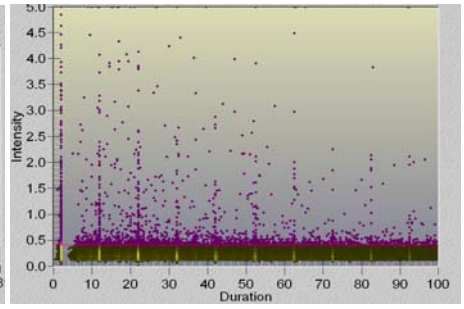


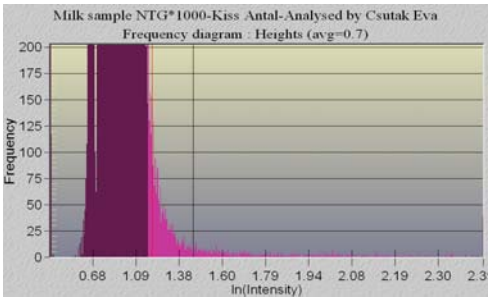


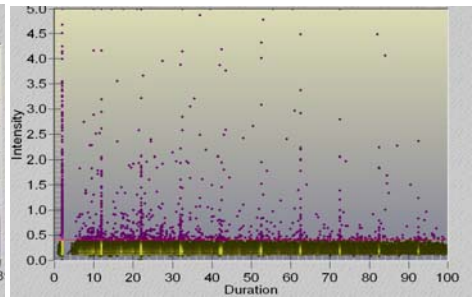
NTG: 91392CFU/ml-sample 3





NTG: 272785CFU/ml-sample 7





NTG: 114321CFU/ml-sample 8

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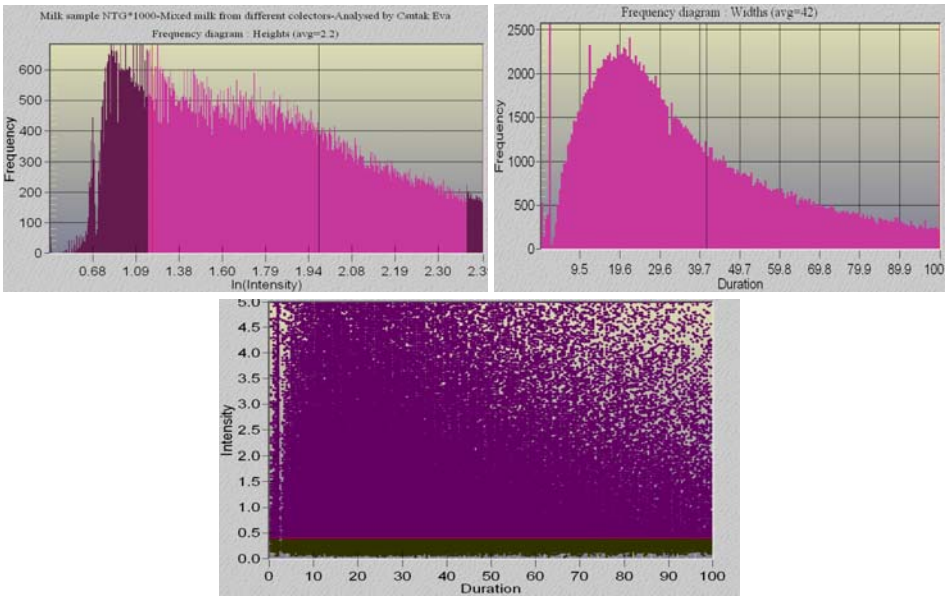


NTG: 8580943CFU/ml-sample 6

1. ábra: A nyerstej csíraszámának meghatározása

Influence of raw milk protein on the pH variation and LA-5 and BB-12 multiplication

Because in Romania raw milk is paid in relation of protein content we were interested on finding the possible positive effects of protein addition to the development of probiotic strains (for example, addition of peptone as a supplement in the media). As it is presented in *Figure 2*, after measurements for 6 hrs incubation, milk protein content of raw milk has minor influence on the pH variations. Same results we had with BB-12 probiotic bacteria, only the acidification process was slower with 3,6%. The requested protein content by EU (3,2%), is enough for multiplication of probiotic bacteria. Protein content of raw milk has importance in obtaining of cheese.

How we can observe from *Figure 2* protein content of milk has no major influence on probiotic bacteria multiplication.

CONCLUSION

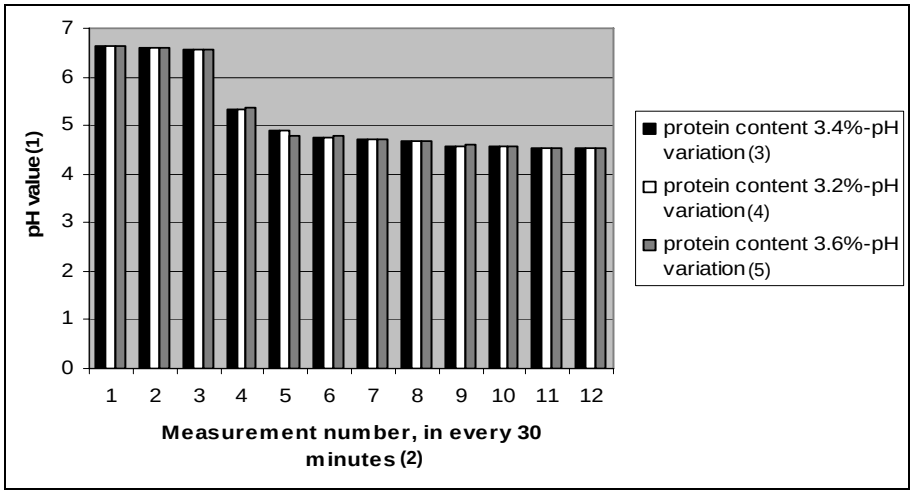
The quality of raw milk in Romania needs to be improved, in order to obtain extra quality probiotic yoghurts. At his moment the number of total bacteria (NTG) exceed the limit accepted by EU legislation (100000 CFU/ml), and it can induce technological problems during production.

In this context, the Gordon Prod company where our experiments were done, tried to investigate the impact of existing NTG in raw and pasteurized milk on LAB probiotics (*Lactobacillus acidophilus* and *Bifidobacterium spp*.) multiplication.

We investigated as well the effect milk proteins on pH and LAB development, the influence of NTG in raw milk before and after pasteurization, on lactic fermentations and LA-5 and BB-12 activities.

Figure 2.

The influence of the raw milk protein on the probiotic LA-5 development and pH values, after 6 hrs of incubation at 38 ºC



2. ábra: A nyerstej fehérjetartalmának hatása az LA-5 bactérium fejlődésére es a pH-érték módosulására

pH-érték(1), Mérés száma, minden 30. percben(2), Fehérjetartalom 3,4%-os pH-változásnál(3), Fehérjetartalom 3,2%-os pH-változásnál(4), Fehérjetartalom 3,6%-os pH-változásnál(5)

We found out that pasteurization at 72 °C was not enough efficient and even after 95 °C pasteurization, the presence of original microorganisms can affect the probiotic development. Small increases of milk total protein content seem not to influence the LABs development.

Generally the BB-12 activities were lower in our experiments comparing with LA-5 and multiplication of both strains was reversely correlated with NTG values at same conditions.

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