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Effect of various prebiotics on LA-5 and BB-12 probiotic bacteria multiplication, and on probiotic yoghurt production

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Abstract. To obtain a good quality probiotic yoghurt the quality of raw milk is a prerequisite condition. 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 (Denmark) and importance of different added prebiotics to milk. Prebiotics are a category of functional food, defined as: Non-digestible food ingredients that beneficially affect the host by selectively stimulating the g rowth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. I studied multiplication of these two probiotic strains without prebiotics, with molasses, lactose and peptone, like prebiotic. I observed, that prebiotics have positive influence on probiotic bacteria multiplication, also their concentration is very important. Added molasses in higher concentration than 2% inhibit multiplication of LA-5 and BB-12, and have negative influence on acidification process and probiotic yoghurt production. From technological point of view, a combination of presented two probiotic strains present many advantages in taste, structure, and acidification process of probiotic yoghurt.

Key words and phrases: probiotic yoghurt, probiotics, prebiotics, survival of probiotic bacteria

1 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*, *Barzoi*, 2002; *Banu*, 2002; *Banu*, *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*, *Martindale*, 2006; *Costin*, *Segal*, 2001; *Macrovei*, *Costin*, 2006; *Tomasik*, *Tomasik*, 2006)

Prebiotics are a category of functional food, defined as: Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health.

This was updated by Roberfroid: "A prebiotic is 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.' Today, only 2 dietary nondigestible oligosaccharides fulfill all the criteria for prebiotic classification." Those 2 are fructo-oligosaccharides and galacto-oligosaccharides. Use of the term other than in that manner is incorrect, since all oligosaccharides do not fit this definition, i.e. mannanoligosaccharides (MOS). They may confer other positive benefits, but are minimally utilized by the comensural bacteria. Typically, prebiotics are carbohydrates (such as oligosaccharides), but the definition does not preclude non-carbohydrates. The most prevalent forms of prebiotics are nutritionally classed as soluble fibre. To some extent, many forms of dietary fibre exhibit some level of prebiotic effect

Traditional dietary sources of prebiotics include soybeans, inulin sources (such as Jerusalem artichoke, jicama, and chicory root), raw oats, unrefined wheat, unrefined barley and yacon. Some of the oligosaccharides that naturally occur in breast milk are believed to play an important role in the development of a healthy immune system in infants, but these are not considered prebiotics, as they do not act through the intestinal microflora. Prebiotic oligosaccharides are increasingly added to foods for their health benefits. Some oligosaccharides that are used in this manner are fructooligosaccharides (FOS), xylooligosaccharides (XOS), polydextrose and galactooligosaccharides (GOS).

Some monosaccharides such as tagatose are also used sometimes as prebiotics. (Food-Info.net Wageningen University). Studies have demonstrated positive effects on calcium and other mineral absorption, immune system effectiveness, bowel pH, and intestinal regularity. Correlations have also been made with other positive health factors, but more research is required. The immediate addition of substantial quantities of prebiotics to the diet may result in a temporary increase in gas, bloating or bowel movement. It has been argued that chronically low consumption of prebiotic-containing foods in the typical Western diet may exaggerate this effect.

Objectives:

a) Preparing of milk samples with prebiotics: molasses, added peptone, lactose,
b) Study of molasses concentrations effect on probiotic bacteria multiplication.
c) Influence of added peptone, and combination of peptone and molasses in different concentration on LA-5 and BB-12 bacteria multiplication.
d) Multiplication of probiotics in molasses and peptone solution directly.
e) Multiplication of different probiotic bacteria in molasses added sterilized milk.
f) Obtaining of probiotic yoghurt by using molasses like prebiotic; LA5 and BB12 like probiotic strains (determination of product's shell-life).

2 Material and methods

2.1 Preparation of probiotic yoghurt with prebiotics

Technology of probiotic yoghurt processing is influenced by many factors, as presented in previous chapters.

The chemical composition of raw milk, the total bacteria number, somatic cell numbers, inhibitors in raw milk; all these factors influence the quality of the final product.

Because it is a probiotic dairy product, not only the sensorial, chemical and biological properties of product are important, but the number of probiotic bacteria after the end of product's shell-life.

How I presented in first chapters the quality of raw milk in Romania is a continuing problem yet. Specially the NTG (number of total bacteria/germ) causes problems at yoghurt production.

For probiotic yoghurt production we need raw milk with good quality parameters:

- acidity: max. 18 T°
- NTG :< 100000/ml
- NCS : < 400000/ml

Because these parameters are not realized every day in dairy factories, in my thesis I tried to find a solution for these situations.

By adding prebiotics to raw milk, specially oligosaccharides, the production time of yoghurt may be shortened, and these prebiotics are nutrients for probiotic bacteria.

I tested inulins and honey, like prebiotic and beet molasses from a sugar producing company.

Specially I tested the molasses effect on probiotics multiplication. Molasses was tested like functional food in different industries, but I wanted to demonstrate, that it may be used not only like feed for animals, but like prebiotic for special probiotic yoghurt. It is a by-product at sugar production, with high sugar content. The extraction process of sugars from beet molasses, after elimination on sucrose is really expensive; that is why it is not applied on industrial level.

Molasses I used for my trials contains 46,5% total sugars, 44,55% sucrose, 0,75% reducing sugars and 1,14% of raffinose. Its protein content was 12,4%. The detailed composition is presented in *Table 1.*

Table 1: The chemical composition of molasses used for trials

	Beet molassescomposition , %
Total Sugars	46.5
– Sucrose	44.55
– Reducing Sugars	0.72
– Raffinose	1.14
Non-Sugar Organic Matter	16
– Nitrogen components as protein (6.25 * N)	12.4
Betaine	4
Glutamic Acid	3.64
– Non-nitrogen bodies	6.92
Soluble gums/other carbohydrates	3.6
Organic acids	3
- Crude Ash	9
Sodium (as Na)	0.32
Potassium (as K)	5
Calcium (as Ca)	0.34

Molasses I used for my experiments is from a sugar producing company.

2.1.1 Production of probiotic yoghurt with molasses solution (different concentrations)

Preparing of molasses solution with different concentrations:

For these trials we used molasses solutions with different concentration: 1%; 1,5%; 2%; 3%; 5%.

Molasses solutions were prepared from molasses. To prepare solution with 1% concentration: in an Erlenmeyer 150 ml we measured (with analytical balance) 1 g of molasses and 99 ml of distilled water. After an energic shaking this solution must be sterilized in heating chamber at 105°C 30 minutes. Before starting the sterilization process the Erlenmeyer must be covered. In Gordon's lab I used Memmert heater (etuva).

After sterilizing with IBCm Bactocount is verified the number of total bacteria from solution. If it is higher than 50 CFU/ml the sterilizing process must be repeated. Do not increase the sterilizing temperature! It is recommended a longer time of sterilization for a better result (molasses contains sugars, what may be affected by a higher temperature, and the concentration may be modified, also).

The number of total bacteria may be determined by inoculation on MRS agar or nutritive agar, also, but this process takes a very long time (72 hours), and in this time the NTG of solution may be modified.

For inoculation of molasses solution we used LA-5 and BB12 strains prepared from freeze dried cultures, offered by Christian Hansen company.

Preparing method of culture is presented at chapter: *Influence of NTG on probiotics multiplication.*

3.33 units of freeze-dried culture has to be used to 19,98 liter of milk or molasses solution (in this situation). By inoculation we add the culture to sterilized molasses solution. After 4 hours of incubation on 38°C we tested the modified NTG (Lactobacillus acidophylus number).

Bacteria number was determined after 1,2,3,4 hours of incubation.

The same steps were followed with the other molasses solutions with: 1% 1,5%; 2%; 3%; and 5% concentrations.

The preparing method of molasses solutions:

- 1% concentration: 1 g molasses + 99 ml distilled water
- 1,5% concentration: 1,5 g molasses + 98,5 ml distilled water
- 2% concentration: 2 g molasses + 98 ml distilled water
- 3% concentration: 3 g molasses + 97 ml distilled water
- 5% concentration: 5 g molasses + 95 ml distilled water

2.1.2 Multiplication of LA-5 and BB-12 probiotic strains in molasses solution with added peptone

The object of trial is to demonstrate if the added peptone may accelerate the multiplication of probiotic bacteria and to study the adequate concentration for this additive to obtain the best results.

The molasses solution preparing method has been presented. Because the 1,5% solution was the most adequate for probiotic bacteria multiplication I used this concentration for next testing.

On same way like molasses solutions we obtained the peptone solutions from special peptone powder (from Sanimed company): to 0,1 g of peptone by adding 99,9 ml of molasses solution to 1% concentration of peptone solution. The molasses solution (1,5% concentration) with added peptone solution in different concentration has to be inoculated with LA-5 strains, and testing the number of total bacteria after 1,2,3,4 hours.

2.1.3 Adding molasses solutions directly to pasteurized milk and its inoculation with probiotics (LA-5 and BB-12)

The aim of experiments was to test if probiotics can multiplicate faster if they are inoculated directly into milk with added prebiotics (molasses) and to verify if molasses in milk may accelerate the multiplication of probiotics.

For testing molasses effect on probiotics (LA-5 and BB-12) we have to choose a milk with good chemical parameters, total bacteria number and somatic cells number.

Milk we used to this trial was tested by IBCm Bactocount before pasteurizing (determination of total bacteria number); by Somatos analyzer (somatic cells number determination) and by Lactostar (chemical and physical parameters determination).

Fat content of milk was standardized to 2,8%. Its protein content was: 3, 38%; somatic cells number: 164000 CFU/ml; total bacteria number before pasteurization: 78000 CFU/ml, after pasteurization on 95°C, 4200 CFU/ml (determined by inoculation).

Samples were obtained by adding molasses solution to milk, incubated at 36°C, respecting the steps of yoghurt processing technology, presented in previous chapter.

Parameters of milk we use for trials:

- Fat content: 3.85%
- Protein content: 3.47%

- Dry substance: 8.55%
- Freezing point: – 0.534°C

Preparing of molasses solution to samples:

To 5 grams of molasses we added 95 ml of distilled water, obtained a molasses solution with 5% concentration. Molasses solution was sterilized in an Erlenmeyer at 105°C, one hour. To 450 ml of pasteurized milk at 95°C we added 150 ml; 5% molasses solution, obtained milk with 1.25% concentration.

This preparing will be repeated one more time.

The samples (600 ml) will be divided in six, and numbered: sample 1 → sample 6.

Samples prepared in second step will be divided in six and numbered: sample 1'→ sample 6'.

Both samples will be inoculated with 0.02 units of culture=0.006g culture/100 ml.

Samples 1 → sample 6 were inoculated with LA-5 culture, sample 1'→ sample 6' with BB-12.

To obtain a more homogeneous solution it is recommended the inoculation before dividing, but in this case must be very careful with hygiene.

All samples were introduced to incubator (36°C). After 1; 2; 3; 4; 5; 6 hours we measured the pH, and in every hour one sample from each group were eliminated.

By introducing the eliminated sample to cooler (2-4°C) the activity of microorganism was stopped.

2.1.4 Preparing of probiotic yoghurt with molasses

Preparation process of probiotic yogurt is presented in chapter VIII. (flow chart). Together with milk protein and milk powder has to be added the molasses solution. It is very important to be added before pasteurizing process.

After preparing molasses solution was sterilized and its NTG (number of total bacteria) determined by presented methods.

Pasteurized milk with added molasses solutions in different concentrations (one sample with molasses 1,5% concentration and 0.1% of peptone) will be cooled till 38°C and inoculated with LA-5 strain (Lactobacillus acidophilus).

All prepared sample must be divided in two parts (min. 50 ml of sample). One of the samples will be inoculated with LA-5, the other one with BB-12. After inoculation we determine the NTG of inoculated samples by inoculation on Petri plates.

Prepared milk with molasses solutions (different concentrations) after pasteurization on 95°C will be inoculated with LA-5 and BB-12 cultures. From every sample (molasses with 1%; 1,5%; 2%; 3%; 5% concentration) one cup will be inoculated with LA-5 and one cup with BB-12. Molasses solution before adding was sterilized, and it was added before pasteurization.

We used milk with 84000CFU/ml, NTG before pasteurization, NTG after pasteurization was 3600 CFU/ml. Its fat content was 3.8%, protein content 3.35%.

After an incubation of samples on 38°C the number of total bacteria has to be determined.

2.1.5 Influence of added lactose content on probiotic bacteria multiplication

Beneficious effect of lactose on acidification process is demonstrated, already. The objective of my trial was to determine the lactose content influence on probiotic bacteria multiplication, and what is the most recommended dosage for probiotic yogurt production. To increase lactose content I used skimmed milk powder from Bucovina company, with the parameters presented in *Table 2*.

Table 2: Parameters of skimmed milk powder added to milk sample

Milk powder "Bucovina"	Protein content %	Lactose content, %	Fat content, %	NTG, CFU/g	Coliform bacteria CFU/g	Salmonella	E.coli Staph. aureus CFU/g
	34	48	1.5	Max.50000	< 10	Abs/25g	Abs.

To do not modify fat content of milk we used for probiotic yoghurt production it was added skimmed milk powder. Milk we used for testing had 4.52% lactose content, and 3.87% fat content. Its NTG = 74000CFU/ml, NCS = 264000 CFU/ml. Milk powder has to be added to milk before pasteurization process, in this way it is possible destroying of microorganisms from raw milk and reconstructed milk.

2.1.6 Testing survival of probiotic bacteria during the shelf-life of product

I compared probiotic bacteria activity in milk without prebiotics, milk with molasses (added molasses to milk directly) and milk with probiotic strains multiplied on molasses substrates).

The aim of trial was to compare the survival of probiotic bacteria in shelf-life of product, to determine the right shelf-life, and to test if addition of prebiotics has influence on survival of bacteria LA-5. NTG was determined by Bactocount IBCm.

3 Results and discussions

3.1 Properties of probiotic yoghurt using molasses as prebiotic

The influence of molasses concentration on LA-5 multiplication is presented in *Table 3*.

Table 3: Influence of molasses concentration on LA-5 multiplication

Molasses solutions concentrations %	NTG of solutions before inoculation CFU/ml	Number of total bacteria after			
		1 hours; CFU/ml	2 hours; CFU/ml	3 hours; CFU/ml	4 hours; CFU/ml
0	18	13000	1620000	2570000	2650000
1	18	13760	1642000	2580000	2670000
1,5	16	13840	1840000	2199000	2479000
2	12	14100	1214000	2178000	2182000
3	17	11200	884000	1460000	1570000
5	19	10750	812000	1260000	1294000

Molasses solutions concentrations %	pH of solution before inoculation	pH after			
		1 hours;	2 hours;	3 hours;	4 hours;
0	7.20	5.82	4.90	4.40	4.40
1	7.20	5.86	4.92	4.44	4.42
1,5	7.21	5.84	4.98	4.51	4.45
2	7.19	6.07	5.17	4.76	4.55
3	7.17	6.34	5.84	5.14	4.82
5	7.16	6.75	6.05	5.62	5.02

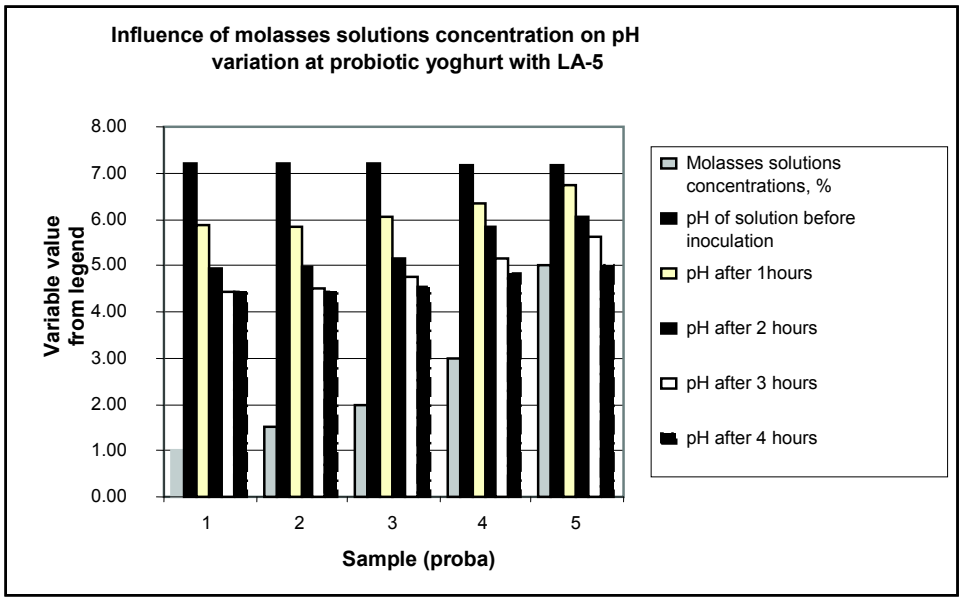

Figure 1: Influence of molasses concentration on LA-5 multiplication

We observe that molasses in 1-1.5% concentration helps the multiplication of LA-5 bacteria. In about three hours the number of total bacteria gets up to maximum level. This is the right time for inoculation of milk with this molasses solution. If we keep bacteria on incubation temperature for a longer time there is a self-inhibition of bacteria. A part of bacteria will be destroyed. If the inoculation of milk is not possible immediately, the inoculated molasses solution must be cooled till 4-6°C. This temperature inhibits the multiplication of bacteria and the acidification of solution.

Higher concentration of molasses solution inhibits the multiplication of probiotic bacteria. So to use molasses as a prebiotic, a 1-1.5% concentration of molasses solution is recommended.

3.1.1 Multiplication of LA-5 and BB-12 probiotic strains in molasses solution with added peptone

Multiplication of LA-5 and BB-12 probiotic strains in molasses solution with added peptone is presented in *Table 4*.

Table 4: Influence of peptone concentration on LA-5 multiplication (original)

Concentration of peptone solution %	Nr. of total bacteria of peptone + molasses solution	N1	N2	N3	N4
0	13570	1960000	2468000	2812000	2822000
0.1	13570	1970000	2476000	2822400	2822600
0.15	13820	1955000	2514000	2842000	2843000
0.2	13100	1953000	2486000	2796000	2798000
0.3	13600	1964000	2416000	2804000	2804000
0.5	13750	1965000	2482000	2614000	2615000

The results of trials are presented in the same *Table 4.* Number of total bacteria is measured in CFU/ml, for inoculation we used LA-5 strain. Concentration of molasses solution was 1,5 %.

N1- Number of total bacteria of peptone + molasses solution after 1 hour of incubation (inoculation with LA-5)

N2- Number of total bacteria of peptone + molasses solution after 2 hours of incubation (inoculation with LA-5)

N3- Number of total bacteria of peptone + molasses solution after 3 hours of incubation (inoculation with LA-5)

N4- Number of total bacteria of peptone + molasses solution after 4 hours of incubation (inoculation with LA-5)

B1- Number of total bacteria of peptone + molasses solution after 1 hour of incubation (inoculation with BB-12)

B2- Number of total bacteria of peptone + molasses solution after 2 hours of incubation (inoculation with BB-12)

B3- Number of total bacteria of peptone + molasses solution after 3 hours of incubation (inoculation with BB-12)

B4- Number of total bacteria of peptone + molasses solution after 4 hours of incubation (inoculation with BB-12)

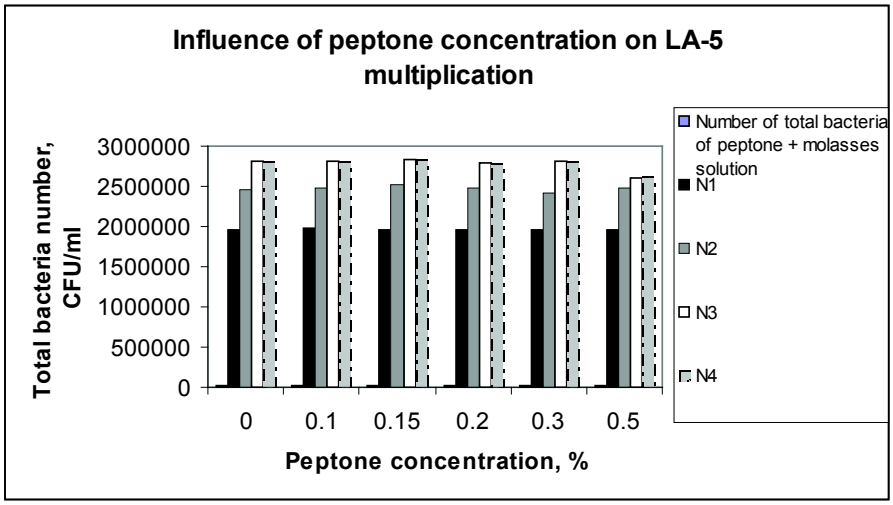

Figure 2: Influence of peptone concentration on LA-5 multiplication

For inoculations we used LA-5 strains in dosage presented in previous trial. The test was repeated with BB-12 probiotic strain. BB-12 strains preparing method was the same like we used to LA-5.

The number of total bacteria of 1 ml of prepared culture is: 13720 CFU /ml. The results of trials are presented in *Table 5.* Number of total bacteria is measured in CFU/ml, for inoculation we used BB-12 strain. Optimum incubation time is 3-4 hours.

Table 5: Influence of peptone concentration on BB-12 multiplication

Concentration of peptone solution %	Nr. of total bacteria of peptone + molasses solution	B1	B2	B3	B4
0	12470	1640000	2173000	2322000	2320000
0.1	12470	1650000	2175000	2322400	2322000
0.15	12810	1645000	2114000	2340000	2340000
0.2	12100	1613000	2085000	2292000	2292500
0.3	12700	1644000	2012000	2200000	2200000
0.5	12450	1647000	2125000	2314000	2312000

Probiotic bacteria are multiplying with different speed. *Bifidobacterium's* BB-12 multiplication speed is lower than LA-5's. The acidification process, measured in pH, is more intensive at LA-5, also. But as common properties we can observe:

Both culture's multiplication were accelerated by molasses solution 1.5%, and by peptone, also. 0.1% of peptone solution helps the multiplication of probiotic bacteria, but the same results were obtained with 0.2, 0.3, 0.5 % added peptone. So added peptone in 0.1% helps the multiplication of bacteria and the acidification process, but a higher concentration of peptone it is not necessary, it would not be useful, only uneconomical.

3.1.2 The influence of molasses on probiotic yoghurt obtained from pasteurized milk inoculated with LA-5 and BB-12

The influence of molasses on probiotic yogurt obtained from pasteurized milk inoculated with LA-5 and BB-12 is presented in *Table 6.* First six saples were inoculated with LA-5, samples 1'-6' with BB-12 strain.

Table 6: Molasses influence on acidification process

Sample/hours of incubation	pH	Lactic acid content%
1	6,69	0.20
2	6,63	0.22
3	5,59	0.21
4	4,70	0.71
5	4,51	0.84
6	4,46	0.92
1'	6,73	0.21
2'	6,08	0.34
3'	5,69	0.43
4'	5,17	0.50
5'	4,99	0.64
6'	4.42	0.94

After 4 hours of incubation from each sample we determined the pH, lactic acid content by photometer, and product's sensorial properties. This concentration of molasses solution was used, because at previous trials we observed that bacteria multiplication was at maxim level if molasses solution's concentration was between 1-2%.

I tried a higher concentration for molasses solution in milk (5%), but as I observed at previous experiments, also, this concentration inhibited the mul-

tiplication of bacteria and another problem was the lactic acid content's determination. At this concentration it was impossible todetermethe lactic acid content of yoghurt, because the sample's color became darker.

Not only colour of product was affected, but its taste, also. So a concentration between 1-2% of molasses has stimulating effect on probiotic bacteria multiplication and doesn't modify appreciably the final product's sensorial properties. The next parameters were determined after 1; 2; 3; 4; hours of incubation: acidity of product; NTG; lactic acid content; pH; taste; sensorial properties. We used the same raw milk for all trials. Dates of trials are presented in table 7.

Table 7: Production of yogurt with molasses-inoculation with LA-5

Molasses solutions concentrations %	NTG of milk before inoculation CFU/ml	Number of total bacteria after 1 hours; CFU/ml	2 hours; CFU/ml	3 hours; CFU/ml	4 hours; CFU/ml
0	3600	18400	1860000	2620000	2615000
1	3600	19760	1963100	2780000	2680000
1,5	3600	19840	1960000	2799000	2779000
2	3600	14600	1414000	2478000	2382000
3	3600	14200	874000	2160000	2160000
5	3600	11850	841000	2044000	2033000

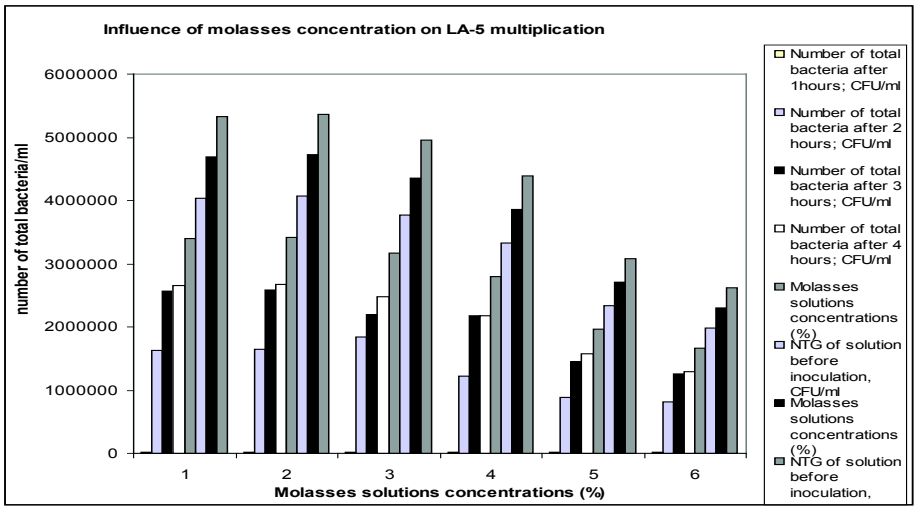

Figure 3: Production of yogurt with different concentration of molasses-inoculation with LA-5

Table 8: Production of yogurt with molasses-inoculation with BB-12

Molasses solutions concen-trations %	NTG of milk before inoculation CFU/ml	Number of total bacteria after 1 hours; CFU/ml	2 hours; CFU/ml	3 hours; CFU/ml	4 hours; CFU/ml
0	3600	18400	1860000	2620000	2615000
1%	3600	15460	1663100	2380000	2384000
1,5%	3600	15840	1660000	2394000	2396000
2%	3600	13500	1463000	2175000	2173000
3%	3600	12200	679000	2060000	2110000
5%	3600	11650	646000	2034000	2133000

By keeping the yoghurt in small sterilized cups it's possible to deduce product's shelf-life. For the determination of product shelf-life we have to analyze some parameters: sensorial properties; biological properties of product and chemical modifications (specially the acidity of yoghurt). Yoghurt samples were tested after 5 days; 10 days; 15 days; 16 days; 17 days; 18 days; 19 days; 20 days. Results are presented in table 9.

Table 9: Modification of LA-5 a number in product shelf-life

Yoghurt/ molasses	Acidity, T°	pH	NTG after (× 1000) days: 5	10	15	16	17	18	20
0%	84	4.42	2670	2670	2670	2660	2660	2630	2580
1%	86	4.44	2680	2680	2680	2570	2560	2470	2300
1,5%	83	4.51	2779	2779	2779	2450	2450	2380	2260
2%	77	4.76	2382	2382	2382	2360	2360	2280	1982
3%	62	5.14	2160	2160	2160	2000	2000	1950	1096
5%	49	5.52	2033	2033	2033	1960	1950	1860	1080

Table 10: Modification of BB-12 a number in product shelf-life

Yoghurt/ molasses	Acidity, T°	pH	NTG after (× 1000) days: 5	10	15	16	20
0%	81	4.50	2370	2370	2340	2340	2110
1%	82	4.52	2380	2380	2350	2340	2130
1,5%	79	4.57	2394	2394	2320	2310	2010
2%	74	4.62	2175	2175	2085	2085	1980
3%	62	5.18	2060	2060	1960	1960	1650

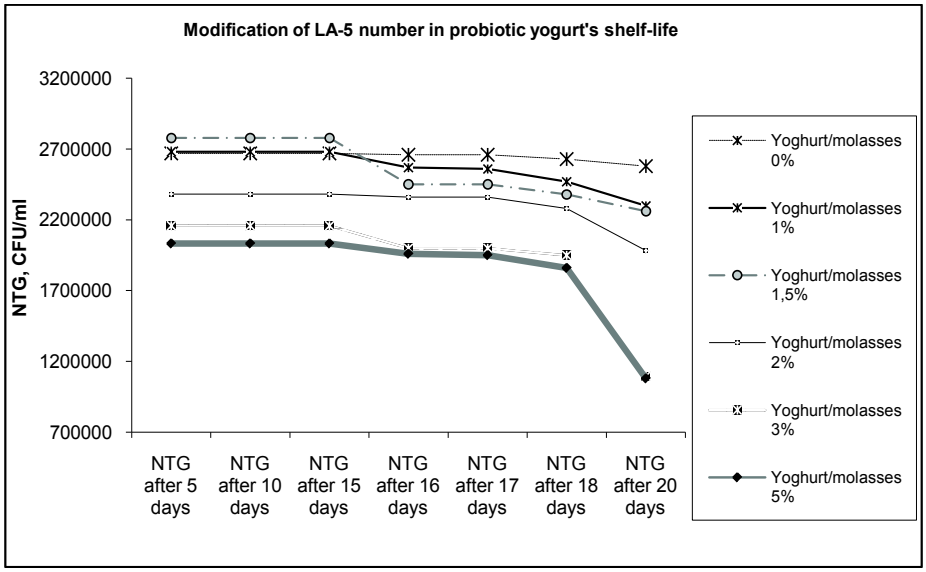

Figure 4: Modification of LA-5 a number in product shelf-life

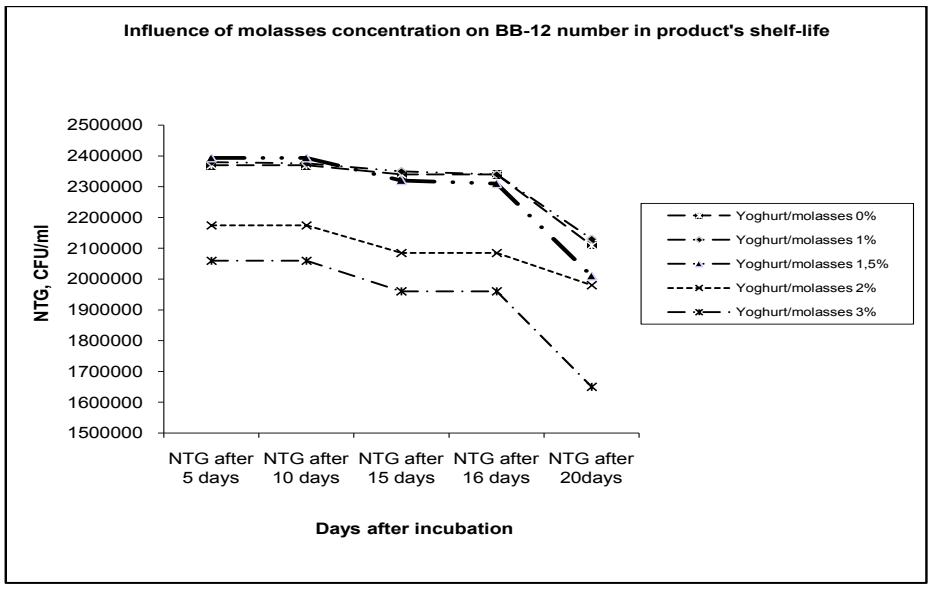

Figure 5: Modification of BB-12 a number in product shelf-life

Added molasses is influencing the multiplication of probiotic bacteria, the acidification process, pH modification and lactic acid content, also. How it was proved by previous trials it is recommended a molasses concentration between 1-2%. By this experiment we tested the molasses concentration effect on post-acidification process. Number of bacteria may not decrease significantly in product's shelf-life, because probiotic bacteria number at the end of product's shelf-life has to be higher than 108 CFU/175g of yoghurt (legislation). After 18 days of storage the number of LA-5 and BB-12 d ecreased considerably. A high concentration of added molasses influenced negative survival of probiotic bacteria. High molasses concentration results in a higher acid content of product, and this acidity destroyed a part of probiotic bacteria. Taste of yogurt with 5% molasses concentration caused a bitter taste, and the color of product became darker.

A commercial and technological advance may be obtained by producing probiotic fruit yoghurt. In this case the taste of molasses in not palpable. Fruits have to be sterilized before using and they have to be added to yoghurt after yoghurt producing, before dosage.

Probiotic yoghurts we obtained may have a shelf-life of 14-18 days. Shelf-life is depending on storage conditions, temperature, and manipulation. Probiotic yoghurt has to be stored at 2-6°C, in clear and clean deposits.

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