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FACULTY OF VETERINARY MEDICINE
SPECIALIZATION HEALTH TECHNOLOGY AND EXPERTISE-
VETERINARY**

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DOCTORAL THESIS

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**RESEARCH REGARDING SOME QUALITY AND
HYGENE RELATED ASPECTS OF VARIOUS
SORTIMENTS OF ACID MILK PRODUCTS**

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ABSTRACT

Milk and its derived products, which are used as such or prepared with other foods, play an important role in a rational and diethetical food diet (menu).

Milk is one of the base food products in our nutrition, due to the fact that it contains all nourishing substances necessary to a normal growth, development and functioning of the body.

The processing of the milk in acid milk products generates some new proprieties, of great relevance, namely: the increase of the nutritional, biological value, the enriching of the products with dietetical and therapeutical proprieties.

From a technological point of view, the diethetical milk products (yoghurt, kefir, sour milk), show a series of phases common to all sortiments, but also some distinct phases which generate certain organoleptic, phisyochemical proprieties individualizing them as a product.

Within the framework of the effectuated studies, one has intended the investigation, the thoroughgoing and completion of the data found in the special literature with new personal observations.

The first part contains a bibliographical study, with data referring to the researched subject, where as in the second part, personal research results are descrined.

The thesis brings up to date information reflecting the present stage of knowledge in the field of quality and hygiene related requirements of the milk raw product which is used in producing acid milk products.

The second part of the thesis, the one containing the personal research results, is structured in 9 chapters, where aspects regarding the quality and hygiene of different sortiments of acid dairy products are described.

The quality and the hygienic level of milk depend on a series of factors related to the health state of the animal, the hygienic conditions of the milking process and of the transportation, on the handeling, and keeping of the milk. From the point of view of its quality, milk must reach first of all the nutritional qualities, especially considering that it forms part of the daily intake of children, of the elderly and even of adults.

From the point of view of the hygiene, milk must be free of any physical, chemical, biochemical and microbiological noxious substance. Considering the above discussed aspects, in the present paper one has had in mind the following major objectives, which are contained in 9 chapters. In chapter 4, one has presented the objectives of the research study, the corpus of materials used and the method of working ; chapter 5 comprises investigations regarding : the measuring of the freshness and of the integrity of the milk as a raw product (from an organoleptic and physiochemical point of view) ; the determining of the pesticides residues, their radioactivity, the contained heavy metals, antibiotics, nitrates and other polluting substances which are contained in milk as a raw product. In chapter 6, one has effectuated microbiological investigations of the milk as a raw product ; in chapters 7 and 8 : microbiological investigations of milk after its pasteurising and bringing it to a certain concentration level ; microbiological investigations of the substances added in order to obtain a better aroma quality and a better nutritional value ; in chapter 9 : microbiological investigations regarding the state of hygiene and the stage of the technological flux ; in chapter 10 : microbiological investigations regarding the quality and purity of the leaven ; in chapter 11 : multiple physiochemical and microbiological investigations regarding the final products at S.C.Multicom, but also from the comercial network where it is distributed; and respectively chapter 12 : bacteriological and micotic investigations of certain sortiments of acid milk products.

The investigations were unfolded in the following locations : S.C. Multicom Grup S.A. Pantelimon ; The veterinarian Laboratory Constanta : The Veterinarian Medical School Iasi.

The physiochemical researches described in chapter 5 were conducted on a number of 120 probes, and the following values were obtained for the effectuated determinations : a 17-20°C temperature, with an average of 18,50°C, a 1,027-1,030 density, with an average of 1,028, total dry substance (TDS) % 11,50-12,90% with an average of 12,45%, skimmed dry substance (SDS) % 8,30-8,98% with an average of 8,67%, fat % 3,3-3,7 with an average of 3,62, acidity 17-20°T with an average of 17,80°T, protein 3,03-3,48% with an average of 3,33%. Comparing the obtained values with the standard admitted limits (1,029-1,030 for the density ;12,2-13,0% total dry substance (TDS) ; 8,5% skimmed dry substance (SDS) 3,6% fat, acidity 19°T, protein 3,3%), the results were located in the range of the admitted limits.

The level of the radioactive contamination in milk as a raw product has registered low values: < 10Bq/l in all effectuated tests on all routes.

The effectuated researches regarding the establishing of the organochlorurate pesticide residues α HCH, β HCH, γ HCH, DDT total and organo-phosphoric, of heavy metals and arsenic were conducted on a number of 160 probes, respectively 120 probes of milk as a raw product. The results of the determinations are presented in fig. 5.7. and no. 5.8.

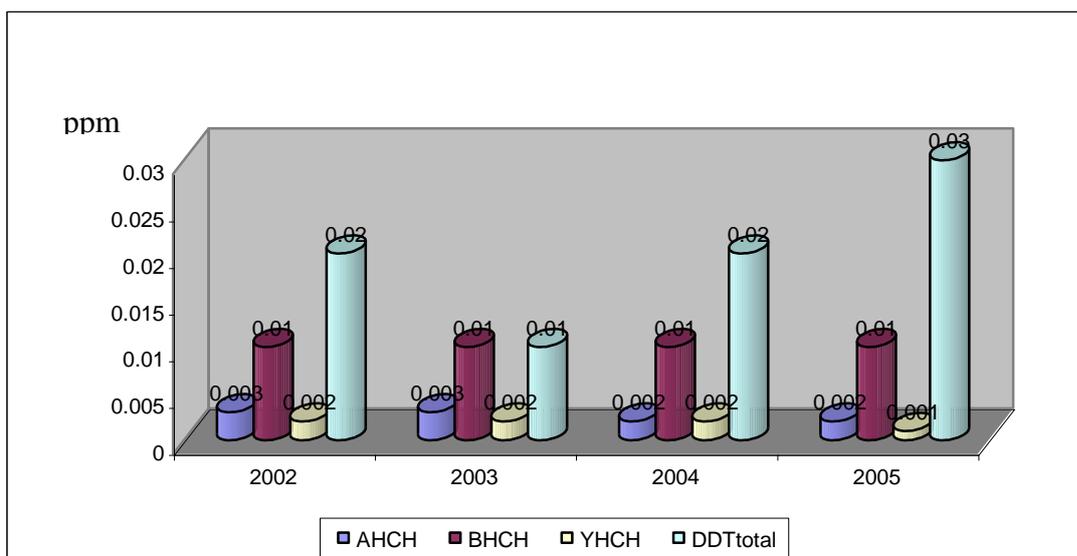


Fig. 5.7. Valori medii ale reziduurilor de pesticide organoclorurate la laptele materie primă
Fig. 5.7. Average (Medium) Values of the Organoclorurate Pesticides in Milk as a Raw Product

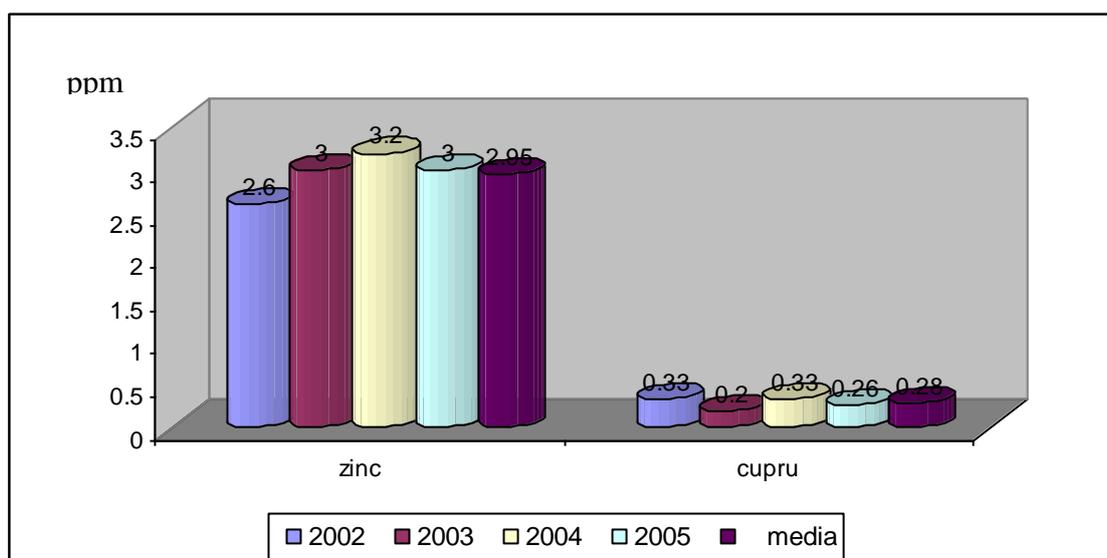


Fig. 5.8. Cercetări privind reziduurile de As și metale grele ale laptelui materie primă
Fig 5.8. Researches regarding the As and heavy metal residues of the milk as a raw product

The interpretation (reading) of the results was conducted taking in account the MAAP/ no.356/2001 stipulations.

After comparing the obtained valued with the standard admitted limits, one has not encountered overpassings of the LMA organoclorurate pesticide residues in the researched milk. Organophosphoric pesticide residues were not detected.

During the entire time of the research, the investigations regarding the presence of antibiotics and nitrates in milk as a raw product did not show any significant relevance.

In chapter 6 one has mentioned microbiological investigations of the milk meant to produce acid milk products, all of these being contained in 3 subchapters.

The researches regarding the determination of the NCS/ml did register values comprised between 25.000-247.000/ml of milk with an average value of 45.500/ml milk.

The analyzed tests were contained in the range of the Ord. MAAP 389/2002 stipulations.

With respect to the microbiological researches regarding the *N.T.G.*/ml of milk from all years, the values were comprised between 150.000-1.290.000/ml of milk, with a total average of 465.400/ml of milk. Some differences appeared probably due to the nonhygienic harvesting of the milk and due to the fact that milk is not cooled down, or not sufficiently cooled down immediately after milking.

In order to establish the efficiency of the quality of the milk as raw product, with regards to a potential pathogenic flora, one has harvested 360 probes, on which 1200 microbiological investigations have been done. The results of the investigations are shown in table no. 6.4

Tabelul 6.4.

**Investigații privind microflora potențial patogenă
Investigations Regarding a Potential Pathogenic Flora**

Anul	Nr. probe	B.colif /ml	<i>E.coli</i> /ml	<i>Campylobacter</i> spp./ml	<i>Staph.</i> cp/ml	<i>Yersinia enterocolytica</i> / ml	<i>Listeria</i> / 25ml	Salm./ 25ml
2002	90	30-34 31	<3	Abs	abs.	abs.	abs.	Abs.
2003	90	21-23 22	<3	Abs	abs.	abs.	abs.	Abs.
2004	90	19-23 21	<3	Abs	abs.	abs.	abs.	Abs.
2005	90	23-30 27	<3	Abs	abs.	abs.	abs.	Abs.
Total	360	19-34 30	<3	Abs	abs.	abs.	abs.	Abs.

The number of coliform bacteria/ml milk, during the entire periode of investigations, reached values between 19-34/ml milk with an average of 30/ml milk. *E.coli*/ml. had values <3 during all the years while researches were done. Compared to the admitted limits by the standard, the average obtained values were normally situated.

The analyzed probes were situated from their bacteriological level in the frame of the stipulations of Ord. M.S. 975/1998.

The microbiological investigations of the milk meant to produce acid and diethetic products after pasteurizing, as well as the concentration level, the adding of additives, aroma (flavour) enhancers, sweateners, fruit pulp, milk powder etc. are described in chapter 7.

The microbiological investigations of milk after its pasteurizing, concentrating, and all the added substances have shown the following aspects:

In pasteurized milk NTG/ml milk, during the entire period, the values were comprised between 3×10^3 - 9×10^3 /ml milk with an average of 5×10^3 /ml milk, an aspect that reveals an efficient pasteurizing.

The number of coliform bacteria/ml milk has registered values comprised between 1,3-9/ml milk, with an average of 2,58/ml milk. Compared to the limits admitted by the standards, the obtained average values were situated normally. In all of the probes, and during all these years, *Salmonella* spp/25ml and *Staphylococcus* c.p./ml. were not identified. The test of the phosphatase and perioxydase came out negative.

From a microbiological point of view, after establishing the quality of the milk after increasing its concentration and adding other substances, the potential pathogenic flora was absent ; the average obtained values in NTGMA/g during all the years while researches were conducted, were comprised between the limits admitted by the standard.

Chapter 8, the one referring to the microbiological investigation of the substances added in order to enhance the quality, the aroma (flavour), the nutritional value, is organized in 2 subchapters 8.1 and 8.2, in which the NTG/g and the NTM/g, the pathogenic and oportunistic microflora levels were measured.

In the case of the products derived from cereals, during 2002, the sulphits reducing bacteria presented values <10 in 2002, 2004, and were absent in 2003, 2005. The NTM/g level had relatively low values, respectively 15 NTM/g, while for 2003, 2004, 2005 the NTM/g was absent. In all other probes (jellies, jams, confitures, fruits, fruit pulp), during the conducted investigations, the NTG/g, NTm/g and bacterial spores/g were absent, and coliform bacteria in products derived from cereals noted values <10 in 2003, 2004, and in 2002, 2005 they were absent. Similarly, in sortiments as jellies, jams, confitures, fruit, fruit pulp, the pathogenic flora was absent. Comparing the obtained values with the limits admitted by the standard one has observed that they were comprised between admitte limits.

The microbiological investigations regarding the hygiene level of the techonological flux are described in chapter 9, along 4 subchapters. The establishing of the NTG/m³ and the NTM/m³ levels was effectuated in order to reache 2 objectives, the determining of the quality of the air in the wearhouse (objective 1), respectively the determining of the air in the working area during ongoing work (objective 2). The results of the investigations are presented in fig. no. 9.1 and no.9.2.

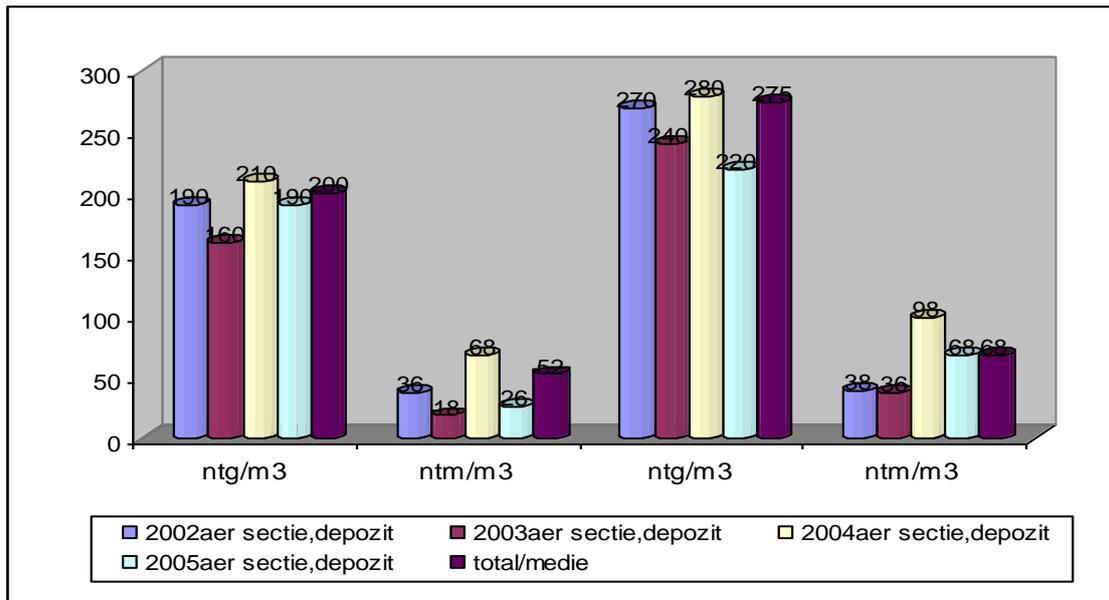


Fig. 9.1. Investigații microbiologice privind starea de igienă a aerului din spațiile tehnologice
Fig.9.1. Microbiological Investigations Regarding the Hygiene Level of the Air in the Techological Areas

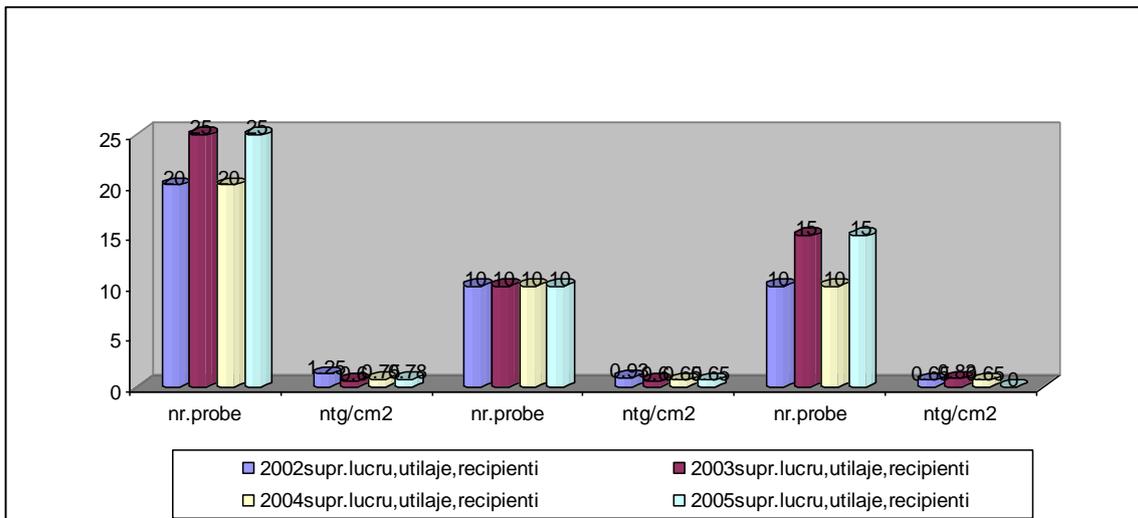


Fig. 9.2. Investigații privind aprecierea testelor de sanitație a suprafețelor de lucru, utilajelor și recipientilor
Fig.9.2. Investigations Regarding the Reading of the Sanitation Tests Done to the Working Surfaces, Machines and Containers

The variation limits of the bacterial charge was situated during the entire period between 200-400, with an average of 275. The NTM//m³ level reached values between 10 and 150, with an average of 68/m³, for the entire period. The average values of the NTG//m³ and NTM//m³ level were comprised between standard limits.

The reasearches regarding the reading of the sanitation tests of the working area, machines and tools, containers, protection equipment, were conducted before work started, and reached an average value of 1,25 N.T.G./ cm², for the working area, 0,93 for the machines and tools, 0,83 for

the containers during the entire period, while the NTG/ cm² was absent in the case of the protection equipment.

The coliform bacteria/10cm² were absent in the working surfaces, on machines, tools, containers, protection equipment during the entire period. These levels were comprised in the frame of standard norms.

The analysis of the data shows that the hygieneizing process in SC Multicom Grup SA Pantelimon is situated between normal limits, and there is a permanent interest in maintaining a clean physical and microbiological environment.

The microbiological investigations conducted in order to establish the efficiency of the level of the purity of the leavens, are presented in chapter 10 and have revealed that coliform bacteria cfu/g, enterococi/g, *Staphylococcus aureus* cp/g reached values <10 for all the years (2002, 2003, 2004, 2005), while the yeast-molds/g, *Salmonella*/25g, *Listeria monocytogenes*/25g were absent during the entire period.

The FD-DSV Chr. Hansen cultures were situated in-between IDF 149 A international standards, with regards to the maximum content of contaminants.

In order to be able to establish the quality of the leavens with reference to the quality and the hygienic level of the used milk and to the quality of the used cultures, physiochemical (acidity related), microbiological determinations were conducted in yoghurt and sana: *bacteriile coliforme/g.*, *Escherichia coli*./g., *Staphylococcus* cp/g., *Salmonella*/25g.

The acidity level in yoghurt reached values of 97°T during the first day, of 110 °T during the 7th day, of 116 °T during the 21st day, and in sana of 131°T during the 1st day, of 134 °T during the 7th day, respectively of 137 °T during the 21st day.

B.coli/g in yoghurt and sana registered values <3, during the first day of the period of safety (safe consume).

The microbiological checking of these products was done in order to identify the pathogenic germs, for the purpose to be able to establish whether the work was done under hygienic conditions, without the contamination of the products with foreign microorganisms, and whether they contain the industrial microflora foreseen by the technology of each separate product. One has studied the presence of the pathogenic bacteria (*bacteriile coliforme/g.*, *Staphylococcus* cp/g., *Salmonella*/25g.) and of the coliform bacteria. In all of the tested products none of the above mentioned categories of microorganisms was identified.

One has analyzed also the number of cells and the surviving of the lactic bacteria during the entire safety period of the product.

The period while lactic bacteria maintained themselves was tested in yoghurt at a concentration level of $8,6 \times 10^{10}$ ufc/g for *L.acidophilus* and at a concentration level of $8,5 \times 10^{10}$ ufc/g in sana, respectively of $9,1 \times 10^{10}$ ufc/g

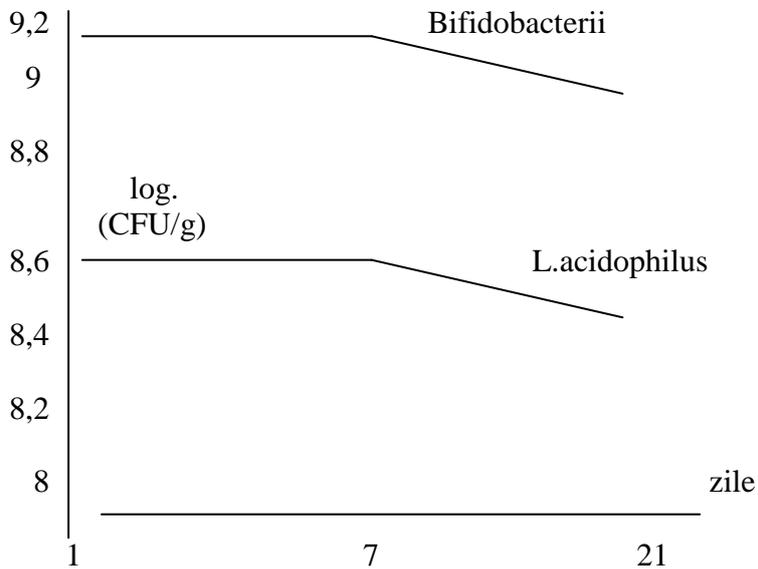


Fig.10.1 Numărul de celule și supraviețuirea lui *L. acidophilus* și a Bifidobacteriilor în lapte fermentat, la 5°C (iaurt)

Fig. 10.1 Number of Cells and the Surviving of *L. Acidophilus* and of Bifide Bacteria in Fermented Milk, at 5°C (Yoghurt)

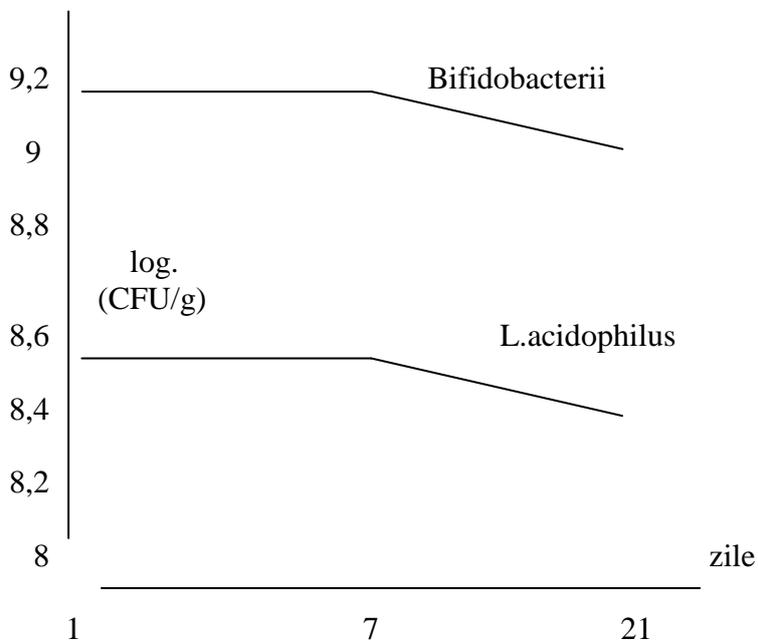


Fig.10.2 Numărul de celule și supraviețuirea lui *L. acidophilus* și a Bifidobacteriilor în lapte fermentat, la 5°C (sana)

Fig. 10.2 Number of Cells and the Surviving of *L. acidophilus* and of Bifide Bacteria in Fermented Milk, at 5°C (Sana)

One has noticed that after a period of 3 weeks, more than half of the bifid bacteria and of *L. acidophilus* were alive, and the number of cells passed by far 10^{10} /ml (fig. no.10.1 and fig. no.10.2).

With the scope of establishing the quality of the leavens depending on the quality and the hygienic level of the used milk, the maintaining capacity of the lactic bacteria during the entire safety period of the product and the survival of the viable cells have been revealed.

In chapter 11, one has conducted investigations regarding the integrity and the hygienic level of the acid milk products as final products, by the means of organoleptic and physicochemical tests effectuated on each sortiment.

The investigations done in order to establish the quality of the products were conducted on three categories of acid milk products (classical products, new products, coagulated and dehydrated products produced for children). Regarding the first category of acid milk products, one has conducted physicochemical tests to five products: natural yoghurt, bioactive yoghurt, diethetical yoghurt, special sana yoghurt, creamy yoghurt. With respect to certain acid milk sortiments, the effectuated determinations included four products: delicious fruit danone, danone Activia musli, danone Actimel and danone Frutissima.

On the coagulated and dehydrated products produced for children one has conducted tests for the following products: danone Danonino Gigantino yoghurt, danone Danonino yoghurt and Activia musli and strawberries. All the acid milk products revealed organoleptic features specific to the sortiments, and the values obtained while the physicochemical test was conducted were situated for all products in between the limits admitted by the standard.

Chapter 12, the last chapter of the paper, presents the microbiological investigations conducted on a number of 300 samples, respectively 75 samples a year, meaning a total of 1500 investigations were effectuated.

The number of coliform bacteria registered during the entire period values >3 for natural yoghurt products and >1 for the following products: bioactive yoghurt, diethetic yoghurt, special sana yoghurt and creamy yoghurt. *E.coli* were absent in all acid milk sortiments. Considering the fact that the number of coliform bacteria shows the contamination degree of milk, one can conclude that the processing of the milk was done under adequate hygienic conditions.

In the case of natural yoghurt and bioactive yoghurt, the coagulazo-positive staphylococcus parameter has registered values >10 . All products were free of pathogenic germs (*Salmonella* spp. and *L. monocytogenes*).

The results of quality and hygiene related requirements of the milk raw product which is used in producing acid milk products from a physicochemical and microbiological point of view were situated for all products in between the limits admitted by the standard.

The present paper presents a high theoretical and practical interest and contains personal contributions presented along the chapters in which certain qualitative and hygiene related aspects of different acid milk products are described.