

ABSTRACT

Key words: microorganisms, milk, quality, food safety

In the past 20 years our country consumers, have expressed interest in healthy eating, becoming the main factor of influence on the new trends emerging food market. Main goal that remains is to improve human health, through better food quality and better control of production in all required health and welfare of animals from which the products are furnished.

It was considered that, once Romania joined the European Union, the food market will evolve to align with European standards by improving the quality of Romanian products. Our country directly applies the new EU hygiene regulations of the primary products of animal origin for human consumption.

Certain foodstuffs may present specific hazards to human health and is necessary to lay down specific hygiene rules. This is especially true food of animal origin which was frequently found microbiological and chemical hazards. These health rules have reduced barriers to trade in those products, which contributed to the internal market development, while ensuring a high level of public health protection. Therefore it is necessary to evaluate continuous progress for improving milk quality raw milk, as restructuring and modernization set for the transition period for dairy livestock and milk collection centres.

Food traceability of is vital for ensuring food safety. Food business operators, which are responsible into an establishment centred to approval subjects under this Regulation should comply with general rules laid down in Regulation (EC) no. 178/2002 (1)

Hygienic status of raw milk is conditioned by some factors, among them we can include: condition of the animals and mammary gland in particular, general hygienic condition of unit, practicing hygienic conditions for manual or mechanical milking, milk hygiene primary conditioning , hygienic conditions of handling, transport, storage of milk. Milk impurities

provides important relationships hygiene in milk collection was realized (hygiene for vessels, milking human operators, in rooms where milk was kept) and on how the milk was handled.

Milk quality is important not only for farmers but also for industry that process milk into products. Its' causes: maximum timing for milk storage, range of products that can be made, products taste, the term of validity of the products, processor production losses.

It is prohibited commissioning consumption of milk which show odours, physical-chemical and bacteriological changes.

According to updated regulations for milk quality standards, hygiene conditions from the producer to reach the mass consumer must be met. Milk that leaves udder cow have a limited number of bacteria (<100/ml), but once exposed to air and contamination microflora of, and hands dirty milking, dirty milking and storage equipment, promotes multiplying and increasing bacteria. Obviously, if the milk is cooled to temperatures below 4 ° C then the rate of bacteria is reduced. Also, milk cells are an indicator of hygienic quality, which is of particular importance. Quantitative and qualitative assessments of milk cells presence are important for understanding many physiological and / or pathological mechanisms in mammary gland. Mastitis negative effects on production, composition and wholesomeness of milk are unanimous recognised, so that new EU regulations focus on somatic cell count, so that EU countries member should take those regulations that are reducing the number of cells per ml milk and so keeping them competitive.

Romanian Government decided by Order 1106/2003 to introduce a few steps to fully realize the compliance requirements:

Phase I 01.01.2005 - 31.12.2006 NTG \leq 1000000; SCC \leq 600,000

Phase II 01.01.2007 - 31.12.2008 NTG \leq 500000; SCC \leq 400,000

Phase III 01.01.2009 - 31.12.2011 (with extension up to 31.12. 2013) in which timing, raw milk must meet hygienic quality criteria's' finally: microbial counts in at 300C (per ml) - \leq 100 000 (geometric average over two months with at least two samples per month) and somatic cell content (per ml) - 400000 (geometric average over a period of three months, with at least one sample per month, unless the competent authority defines another methodology to take into account seasonal variations in production levels.

Food business operators responsible for raw milk production holdings, as raw milk distributors for processing units, through milk collection centres, must meet a number of conditions and obligations. All these are included in a National Program for quality of raw milk

from cows improving, implemented by Central Veterinary Authority at Order of both ANSVSA Romania and MAFRD.

However, experts ANSVSA assessments to the end of European Commission report showed that at the end of the transitional period granted to Romania, December 31, 2011 - a rate of 19% of raw milk delivered to processing units, can not achieve compliance plans with quality parameters (NTG / ml, and SCC / ml).

Thus, our research focused on: an analysis of the microbiological quality of milk during its traceability, quality assessment of raw milk hygienic quality, by two parameters: total mesospheric aerobic germs in plate count (NTGMA/ml) and somatic cell count (SCC/ml), according to EU norms and hygiene assessment of the effectiveness of inputs in contact with milk by microbiological tests sanitation.

The paper entitled "Research on improving the microbiological quality of milk to meet the European Union requirements contains 180 pages was written in eight chapters and is structured in two parts as it is required.

The first part (Chapters I, II, III) in 36 pages extension, represents 20% of thesis and summarizes the main data from the literature on the microbiology of milk, hygienic quality of milk in accordance with the European Union, and the main elements of "National Program for improving the quality of raw milk cow, and actions calendar to achieve compliance with Community law ".

Second part (Chapters: V, VI, VII, VIII) in 144 pages extension, that represent a 80 % from personal research during 2006-2009. Each chapter is structured in subchapters that are concerning labour materials and methods currently used, the results and their discussion finalised with partially conclusion.

In the human- food- microorganism's biological system, microbial determinism had a high technological importance, socio-economic, with hygienic implications, conditioning human health. Cow milk quality and early detection of udder diseases, or other conditions that cause lactation disturbances, will continue to remain a major goal for livestock professionals.

In **Chapter IV**, entitled "**Purpose and research objectives**" our research objectives are pursued: an analysis of the microbiological quality of milk during its traceability, raw milk hygienic quality assessment by quality two parameters: total count in number of mesospheric aerobic germ (NTGMA/ml) and number of somatic cells (SCC/ml), according to EU norms, and hygiene microbiological tests sanitation assessment of the effectiveness of inputs in contact with milk.

In Chapter V, entitled "**Research on the microbiological quality of milk during the trace (from the mammary gland to the raw milk manufacturing unit)**" are presented the results of investigations carried out on 688 milk samples from several points, considered by us, critical, that means: milk samples, collected in people domestic units, from bucket, obtained by manual milking cases, milk samples obtained from milk collection tank using milking devices, related with collection points on certain specific routes and milk sample collection from a milk processing receiver center.

From these measurements it was found that of 688 samples of milk microbiology investigated during those four years, 108 (15.69%) samples exceeded the permissible limits of normal NTGMA / ml standard in effect for that period, 36 (5.23%) milk samples were irregular on coliforms microbiological criterion / ml, 9 (1.3%) milk samples exceeding criterion limits for *E. coli*/ml, and 5 (0.7%) samples were identified coagulase-positive staphylococci. It is noteworthy that there were no samples of milk contaminated with pathogenic microorganisms, i.e. *Salmonella*, *Bacillus cereus* and *Listeria monocytogenes*.

Synthesizing data obtained shows that the microbial load of milk collected by manual milking (in bucket) was significantly higher compared with the milk collection tank came from automatic milking.

Thus, inadequate samples of milk collected by manual milking system were in 2006 to 21.7% in NTGMA/ml, 23.17% in coliforms, 4.8%, with *E. coli*, 12.2% spores of anaerobic microorganisms gas-producing, 3.6% contaminated with coagulase-positive staphylococci; in 2007 - 31.6% to NTGMA/ml, 8.33% for coliforms, *E. coli* 1.66%, 16.6% spores anaerobic microorganisms gas-producing; in 2008 -29.26% from NTGMA/ml, 17.1% for coliforms, 4.87% with *E. coli* 9.7% spores anaerobic microorganisms gas-producing, 4.87 with coagulase-positive staphylococci; in 2009 -12% to NTGMA/ml, 4% in coliforms, 4% with *E. coli*, and 8% with spores anaerobic microorganisms gas-producing.

In the case of milk collected by automated system, inadequate milk samples were in 2006 to 19.8% in NTGMA/ml, 2.1% for coliforms, *E. coli* 1.5%, 12.5; of spores anaerobic microorganisms gas-producing, in 2007 - 2.1% NTGMA/ml, 14.5% spores anaerobic microorganisms gas-producing, -2.1% in 2008 to NTGMA/ml, 2.1% spores anaerobic microorganisms gas-producing, in 2009 -2.1% to NTGMA / ml.

Inadequate milk samples collected in the processing units were: in 2006 for NTGMA/ml indices at 50% for coliforms at 8.33% in 2007 at 20.8%, in 2008 -20.8 % to NTGMA/ml, in 2009 - 4.16% to NTGMA/ml.

In Chapter VI, entitled "Research on the implementation of European quality standards for milk processing " are presented the results of those two milk quality parameters: total aerobic mesophilic germs/ml (NTG/ml) correlated with the number of somatic cells/ml (SCC/ml), which were completed with bacteriological examination presented in the previous chapter, that is a guarantee of quality and healthy state and, until today, couldn't yet replaced by other methods of monitoring. Milk sampling preservation during 2006-2009, in order to perform these laboratory tests, was carried out upon law requirements. Daily testing is not economic, so that random samples were taken at irregular intervals. Since laboratory assessment costs are high, numbers of samples and test methods chose were limited in time as number.

Comparative analysis shows that milk from A1 collector center was: in 2006, for NTGMA / ml indices, with adequate monthly average values, in the months I, II, III, IV, IX, X, XI and XII and inadequate months V, VI, VII, VIII, and for NCS / ml indices, with adequate monthly average values, in the months I, II, V, VI, VII, VIII, IX, X, XI, XII and inadequate in months III and IV. In 2007, for NTGMA / ml indices, monthly average values, were adequate in the months I, II, III, IV, V, IX, X, XI and XII and inadequate months VI, VII, VIII, and for NCS/ml indices, monthly average values, were adequate in mostly months except March. In 2008, for NTGMA/ml indices, all monthly mean values, were adequate except for the eighth month, and for NCS/ml indices, all monthly mean values, were within the veterinary rules. In 2009, for NTGMA/ml indices, all values, exceeded the monthly average limit, and for NCS/ml indices, all monthly mean values, were adequate, with the exception of the sixth month.

In A1 collector centre, annual mean values, for NTGMA/ml indices, were: in 2006 at 966.33×10^3 cfu/ml, in 2007: at 500.83×10^3 cfu/ml cfu 2008: $371,67 \times 10^3$ / ml and in 2009 at 320.83×10^3 cfu/ml. For NCS/ml indices, average annual variation was linear in high, with similar values. So, in 2006, annual average was at 224.71×10^3 cells/ml, in 2007 at 202.50×10^3 cells/ml, in 2008 at 163.23×10^3 cells/ml and in 2009 at 182.79×10^3 cells / ml.

Following milk analysis from the collector center A2 it was found that, in 2006, for NTGMA / ml indices, monthly average values were adequate in the months I, II, III, IV, V, X, XI and XII and inadequate months VI, VII, VIII and IX and for NCS/ml indices, all monthly mean values were adequate except February. In 2007, for NTGMA/ml indices, mean monthly months were adequate in I, II, III, IV, V, VI, X, XI and XII and inadequate for months VII, VIII, IX, and for NCS/ml indices, monthly average values, were adequate in the months I, II, III, IV, V, VII, IX, X, XI and inadequate for months VI, VIII and XII. In 2008, for NTGMA/ml indices, all monthly mean values, were adequate except for the seventh month, and for NCS/ml indices

all monthly mean values, were adequate within the veterinary rules. In 2009, for NTGMA/ml indices, monthly average values, were adequate in the months I, XI and XII and poor in other months, and for NCS/ml indices, all monthly mean values, were adequate.

In collector centre A2 annual average for NTGMA/ml, were: in 2006, at 1060 x103 cfu/ml in 2007 at 500.83 x103 cfu/ml in 2008 at 451, 67x103 cfu/ml and in 2009 at 173.83 was x103 cfu/ml. For NCS/ml indices, the average annual values were, ranged from 288.33 x103 cells/ml in 2006, 263.92 x103 cells/ml in 2007, 192.67 x103 cells in 2008 to 140.89 x103 cells/ml in 2009.

Milk from the collector center B1 in 2006 was for NTGMA / ml indices, with monthly average values adequate in the months I, II, III, IV, X, XI and XII and inadequate in months V, VI, VII, VIII and IX and for NCS / ml indices, all monthly mean values were adequate. In 2007, for NTGMA/ml indices, monthly average values were adequate in the months II, IV, V, VI, VII, VIII, X, XI and XII and inadequate in months I, III, IX, and for NCS/ml indices, all monthly mean values were adequate except month VIII. In 2008, for NTGMA / ml indices, monthly average values were adequate in the months I, II, III, VI, VII, VIII, IX, X, XI, XII and inadequate in months IV and V and for NCS/ml indices all monthly mean values were adequate within the veterinary rules. In 2009, for NTGMA/ml indices, monthly average values were adequate in months III, V, VII, IX, X, XI and XII and XII inadequate in other months, and for NCS/ml indices, all the monthly average were adequate.

In the collector center B1, annual average for NTGMA/ml, were: in 2006 at 1445x103 cfu/ml, in 2007 at 464.88 x 103 cfu/ml, in 2008 at was 288.33 x103 cells/ml, in 2007 at 238.00 x103 cells/ml, in 2008 at 196.25 x103 cells/ml and in 2009 at 159, 75 x103 cells/ml.

Milk from the collector center B2 was: in 2006, for NTGMA/ml indices, all monthly mean values were adequate except months VII and VIII, and for NCS/ml indices, all values were adequate. In year 2007 monthly average, for NTGMA/ml indices, all monthly mean values were adequate except months VII and VIII, and the indicator NCS/ml, all monthly mean values were adequate except during IV. In III and 2008, the indicator NTGMA/ml, all monthly mean values were adequate except months VIII and X, and for NCS/ml indices, all monthly mean values were within the sanitary rules. In 2009 for NTGMA/ml indices, monthly average values were inadequate in months V, VII, and X, and other average values fits into the legal limits, and for NCS/ml indices, all monthly averages were adequate except for the third month.

In collector centre B2, annual average for NTGMA/ml indices, were: in 2006 at 897.5 x103 cfu/ml, in 2007 at 423.33 x 103 cfu/ml, in 2008 at 360.83 x103 cfu/ml and in 2009 at 132

x103 cfu/ml. For NCS/ml indices annual average was: in 2006 at 288.33 x103 cells/ml, in 2007 at 282x103cells/ml, in 2008 at 162.42 x103 cells/ml, and in 2009, with a slight increase at 194.75 x103 cells/ml.

Significance statistical analysis of using Fisher's exact test performed on all collector centres, as it shown by NTGMA/ml indices of milk quality presents distinct significant differences ($F > F5\% > F0\ 1\%$) in 2006, 2008 and 2009. Statistical analysis for NCS/ml indices in milk samples collected from four collector centres, showed significant differences ($F < F5\%$) for 2007 and 2009 and significant differences ($F < F5\% < F0, 1\%$) in 2006 and 2008.

Student test showed that for NTGMA / ml indices in 2006, there were significant differences between the mean average of samples and standard media between collector centres A1 and B2, A1 and A2, A2 and B2. In 2008, there were no significant differences between the centres regarding the annual average mean at centres A1 and B2, and in 2009 were insignificant differences in annual mean average between centres B1 and B2. Student test did not apply for the SCC / ml indices, because in Fisher test there were no significant differences found.

In **Chapter VII**, entitled "Research on the effectiveness of sanitation through microbiological control" microbiological investigations were performed: 120 sanitation tests on working surfaces and contact, 120 tests on the surface of protective equipment and clothing milking workers, 100 tests on surface of working tools, equipments and vessels of milking, 120 sanitation tests on the palmers surface of the farm and domestic workers, 40 air microbiota tests of shelters and associated dairy stables milking in cows farms.

During 2006-2009 years, sanitation values for NTGMA indices varied significantly, and it was observed an inadequate dynamic decreasing of percentage in mostly samples analyzed.

For NTGMA indices, were found in 2006, 35.4% adequate samples and 64.6% inadequate samples, in 2007, 61.5% adequate samples and 38.5% inadequate samples, in 2008, 84.6% adequate samples and 15.4% inadequate samples in 2009, 95.4% adequate samples and 4.6% inadequate samples.

For *Enterobacteriaceae* indices: in 2006 were found 73% adequate samples and 27% inadequate samples, in 2007 - 83.5% adequate samples and 16.4% inadequate samples, in 2008 91.7% adequate samples and 8.3% inadequate samples and in 2009 to 97.6% adequate samples and only 2.4% inadequate samples.

For coliforms/ml or/10 cm² indices: in 2006 to 78.8% adequate samples and 21.2% inadequate samples, in 2007 - 91.7% adequate samples and 8.3% inadequate samples, in 2008-95, 3% adequate samples and 4.7% inadequate samples, in 2009 to 100% adequate samples.

In samples from workers' hands there were not isolated coagulase-positive staphylococci and bacteria of *Salmonella* genus. Data analysis obtained by air determination of yeasts and moulds/m³ in animals shelters showed an incidence at 60% for inadequate samples in 2006-2008, that decreasing in 2009 to 40% for inadequate samples. Data analysis in 2006-2009, for microbiological evaluation of production tools, located on the milk route tracing, gives us reflection that is needed to respect and improve hygienic conditions.

Chapter VIII summarizes the main results obtained from research assessments, summarized 9 in as final conclusions.

The paper presented 30 tables and 64 figures and the bibliography includes 143 titles.