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# **RESEARCH CONCERNING THE QUALITY AND THE HYGIENE OF BALKAN CHEESE**

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## **ABSTRACT**

The dissertation entitled, **“RESEARCH ON THE QUALITY AND HYGIENE OF THE BALKAN CHEESE”** is based on the need of current data on the quality of milk and dairy products, on the need to be aware of the current problematics regarding the enforcement of the legal framework and involvement of the raw material milk processors in order to obtain dairy products, hygienic, toxic-free Balkan cheese, respectively, of a trophically outstanding quality.

The dissertation consists of a number of 415 pages and it is structured in two parts; the bibliographical references extending on a number of 107 pages and the own research study extending on a number of 308 pages, and ends in the discussion of the results achieved, drawing of conclusions, submission of proposals and presentation of the images in the appendixes, some of them rather significant for the history of milk processing into Balkan cheese and others relevant to the research conducted.

The presented data are supported by a number of 122 tables, 57 charts, 43 figures and 29 appended images, relevant to the conducted study.

Each individual chapter ends in its own partial synthesized conclusions and in general conclusions, respectively. The bibliographical references gather 282 titles from the national and international literature, internet scientific papers, data from own scientific works and legislation specific to food safety.

The first part of the thesis consists of the introduction, briefly describing the history of Balkan cheese manufacture and the Current awareness stage on the quality and hygiene of the Balkan Cheese with a number of 5 chapters and 19 subchapters furnishing information from the specialized literature and provided for by the current legal framework on the dissertation topic, data which have been used in the interpretation of the research results.

The first chapter shows data on *„The main characteristics of the raw material milk used in the manufacture of the Balkan cheese”*, with aspects referring to the psycho-sensorial analysis of the quality physicochemical, freshness, hygienic-sanitary and microbiological factors of the cow and sheep milk.

The second chapter, entitled, *“The technology of the Balkan cheese manufacture from cow and sheep milk in industrial system and hygiene of the technological flow”* presents, based on the referred literature, the main industrial technologies and traditional flows, assessment of the induced risks in various stages of the technological flows and, eventually, assessment of chemical and bacterial clean condition within the plants for milk processing into Balkan cheese.

Thus, current data in the national, international specialized literature and in the European

legal framework are shown with reference to:

- the technology of Balkan cheese manufacture in industrial system;
- Conventional method of kettle processing;
- Technology of Balkan Cheese manufacture from cow milk with albumin embedding;
- Feta Cheese;
- The technology of Balkan cheese manufacture from sheep milk;
- General hygiene within the plant; hygiene of technological flow and incoming risks in the various stages of the technological chain;
- Hygiene requirements on the chemical and bacterial clean condition;

The third chapter, entitled, “*The technology of Balkan Cheese manufacture in household-artisanal system from cow and sheep milk*” specifies:

- Artisanal methods used in order to obtain Balkan Cheese from cow and sheep milk;
- Hygienic-sanitary requirements enforceable in the artisanal process of Balkan cheese manufacture.

The fourth chapter, entitled, “*Assessment of the food value, of the quality and hygienic condition of the cow and sheep Balkan cheese*” refers to:

- Assessment of the Balkan Cheese quality and food value;
- Assessment of the hygienic condition of the cow and sheep Balkan cheese.

The fifth chapter describes “*The main flaws, degradations and alterations of the cow and sheep Balkan cheese*”.

The second part „*Personal contributions*”, consists a number of 7 chapters with 34 subchapters and 22 items, presenting the topic motivation, research objectives, working material used in compliance with the working methodology and techniques provided for by Romanian ISO Regulations (SR ISO) or the European regulations accepted by ISO (SR EN ISO):

**Working material** – The investigations made process the data achieved 2000 to 2004, on the quality and hygiene of the raw material milk on an initial number of 1576 samples of industrial processed raw material milk and the data achieved 2002 to 2006 for 379 sample of artisanal processed raw material milk, numbers undergoing changes depending on the conducted analyses or the technical criteria.

The working material consisted of relevant samples of milk, Balkan cheese, brine, sanitation tests on working surfaces, tools, equipment, microbial aeroflora tests and water testing from water supply network or own water supply (*1576 matrixes consisting of industrial processed raw material milk samples, 379 artisanal processed raw material milk samples, 228 raw material milk samples were analyzed in accordance with the preventive actions of the*

*quality systems, by rapid methods of finding incipient mastitis, 503 sanitation tests on working and contact surfaces, microbial aeroflora of the industrial technological enclosures, 115 sanitation tests for the traditional units and artisanal manufacturers, 936 sanitation tests drawn intended for self-control, according to the H.A.C.C.P. system requirements, 149 water samples from the water supply network or own sources, reporting the results achieved under the requirements of STAS 1342/1991, working method provided for in STAS 3001/1991 and 135 water samples, 98 from industrial sources and 39 collected from sheep pens in order to assess the water hygienic quality by microbiological analysis, according to SR EN ISO 9308-1/2004, SR ISO 8199/2005 and ISO 6887-1/2002, by reference to Law 458/2002 amended by Law 311/2004 on water drinkability) and on 129 preservation brine samples and on 1730 cottage cheese samples).*

Another series of matrixes subject to microbiological testing consisted of sanitation tests and samples of finished product, drawn simultaneously and mainly from similar surfaces in processing plants with food safety and quality certification systems and plants in which these systems are either in course of implementation or non existent.

The raw material milk under analysis originated in some households of small farmers, holders of milk animals, from the milk collecting plants and from the plants of milk processing into Balkan cheese, Balkan cheese finished product matrixes come from the milk processing plants on the district territory, *with a major percent drawn within the quality self control programmes, from the agri-food markets – from the small artisanal manufacturers.*

The annual fluctuation of the improper samples percent does not strictly show an evolution or involution of the psycho-sensorial parameters, as the reporting periods are different, respectively 2000 to 2004 for the industrial sector and 2002 to 2006 for the artisanal sector, collection of samples and performed analyses had a randomized character and the existent units have always been the same.

The improper milk samples, analyzed during the hot season, from the industrial sector are 0,98% lower for the color, aspect, consistency parameters while the assessment of the taste – smell parameter highlights a percent of approximately 6,5% for the improper raw material milk samples used in the industrial processing of the cottage cheese;

Regarding the milk quality, the highest percent of improper results achieved pertains to the industrial processed cow milk, thus, the impurification degree was of 70,82 % for the good milk category (1), the acidity, during the hot season, was of 11,26% and for density only a percentage of 42,3% from the cow milk samples had a relative density compliant with the regulations in force;

The values achieved for the G% parameters were higher, compared to the reference values provided for in STAS 2418/98, in the cold season, within 3,41 – 4,07% with an average of 3,72% for the cow milk and within 6,4 – 7,01 % with an average of 6,84% for the sheep milk. The abnormal situation of the fat percentage owed mainly to the fat extraction, the milk subject to analysis being, most often, collected in improper conditions, without an efficient homogenization.

The values of the lactose percentage integrate the samples in the two processing systems, industrial and artisanal collected at a percentage similar to that expressed in the literature both for the cow milk and for the sheep milk samples, with a low variability percentage.

For proteins it was found that in the industrial processed sheep milk matrixes a percentage strictly inferior to the limit provided for in STAS 2418/98 for the samples collected in the hot season, the percentage of the protein substances having increased only for the somatic cells-rich milk samples;

The nonfat dry substance SUN % was within the limits provided for in STAS 2418/98 for both types of war material milk, the existent variations were seasonal, due exclusively to the type of fodder providing.

For the freezing – point depression, values within the reference values of -0,54....-0,56 for the cow milk and of 0,57 - 0,548 for the sheep milk were achieved.

The radiometric measurements of the sum of isomers CS<sub>134</sub> and CS<sub>137</sub>, respectively, the assessment of the OCl or OP residue contamination degree as well as the detection of the arsenic or of heavy metals Zn, Cu, Cd, Pb traces highlighted that in Iasi County the fauna, the flora and the waters are not contaminated.

The investigations of the antibiotics residues highlighted that 28,09% (8 cow milk samples and 3 sheep milk samples) of the total collected milk samples showed a positive result, given the non observance of the milking rest period consequently to the administration of drug treatments, there were not any cases of volitional antibiotic add-ons (as preservative) intended to commit fraud.

Assessment of the microbial load based on a number of 10.100 microbiological analyses and leads to the conclusion that the milk, collected and artisanally processed, is more improper than the industrial processed milk, from the point of view of the incidence of the bacterial species with pathogenic potential *coliform bacteria*, *Coagulase-positive Staphylococcus*, *Bacillus cereus*, *Salmonella spp.*, *Listeria spp.*

Thus, the presumptive, which was considered the greatest microbiological load, which exceeds the maximum number of UFC / ml or were produced, was in the milk industry for the

NTG / ml considered as high as 7.3% for the *Escherichia coli* / ml with 0.29 and 7.56% for gas anaerobic bacteria - evidence Wienziril unless item processed milk and 4.94% higher for the coliforms / ml, 1.7% for *Staphylococcus coagulazo-positive*, 0.6% for *Bacillus cereus* (sheep milk), with a rate of 0.48% of the samples of cow's milk and a rate of 0.4% of samples from sheep's milk *Salmonella spp.* parameter and 2.7% for *Listeria spp.* when processing crafted than in industrial processing.

For NCS/ml, the lowest percentage, of 48,32% of proper samples with values below 400.000 was achieved for the industrial processed raw material milk samples, mainly due to the large number of animals from which the milk is collected, to the failure to timely find the incipient mastitis and mixing the satisfactory milk with the milk originated in the animals with mastitis evolution.

The microbiological control of the working surfaces, equipment, instruments and protective equipment highlighted the highest percentage of improper sanitation tests, of 32,17% for the working surfaces assessed by the determination of the  $NTG/cm^2$  parameter for the collections performed in the artisanal processing sector, of 8,43% for the tests assessed for the  $NTG/ml$  parameter capacity in industrial system, of 29,56 for the *coliform bacteria/cm^2* parameter in case of assessing packaging surfaces – plastic containers, polyethylene foils used in artisanal processing, of 19,82 % for the *coliform bacteria/1ml* parameter, for the microbiological examination of the hands of the personnel in the industrial system. The air microbiological examination in the working and storage spaces – of the microbial aeroflora was determined, mainly in the units which industrially process the milk, thus a higher percentage of improper tests was obtained, of 17,70% for the  $NTG/m^3$  capacity, aspects due to the absence of efficient decontaminants, of improper working surfaces from the texture point of view, with artisanal processing or failure to observe the decontaminating programmes in case of industrial processing units.

For the cottage cheese finite product samples, the percent of the improper matrixes percentage pertained to the fresh sheep cottage cheese, having been assessed to 22,72 %.

The psycho-sensorial analysis of the cow cheese preservation brine highlighted the higher percentage achieved by the improper samples of the sheep cottage cheese preservation brine of 20,75%, this being 10,23 percentages higher than the cow cheese preservation brine percentage.

The improper maximum percentage for Gr/SU% of 31,81% reported to the product quality was assessed for the fresh sheep cottage cheese.

The maximum samples percentage assessed as improper for  $H_2O\%$  pertained to the sheep matured cottage cheese samples by 78,57%.

The assessment of the NaCl% highlighted a maximum percentage of improper samples of 11.88% for the 2<sup>nd</sup> quality fresh cow cottage cheese.

The assessment of the NaCl% for the cow cottage cheese preservation brine highlighted a higher percentage of 8,86% for the fresh cheese brine of the total analyzed samples for each individual category.

With regard to acidity determinations (Thörner degrees) for the cottage cheese preservation brine, the highest average was recorded for the matured sheep cottage cheese preservation brine of 220,62% °T.

The conducted research intended for establishing the value of the nitrites residues from the cottage cheese completed by reporting low average values with an average higher than 0,401 ppm for fresh cottage cheese, level which does not influence the products' characteristics and which do not have a toxic effect.

The highest bacterial loads were determined in the sheep fresh cottage cheese, thus for the *Coliform Bacteria* parameter, the obtained percentage was of 29,41 %, for the *Escherichia coli* bacterial parameter was of 29,41% and for *Yeast and Mould* of 41,18% aspects due to the influence of the milking and processing conditions and particularities of the sheep milk.

The research intended for the identification of the matrix contaminations with coagulase-positive *Staphylococcus spp.* allowed for the assessment of the fact that the cow cottage cheese shows the highest improper samples, respectively of 9,30 % for the fresh cottage cheese category, percentages explained by non observance of the finite product preservation conditions.

*Salmonella spp.* showed an improper percentage load higher for the fresh sheep cottage cheese, thus accumulating a percentage of 5,89%, applicable by the processing manner, characteristic to the sheep milk (*S. abortus ovis*).

The *Listeria spp.* microbiological parameter was identified in the highest sheep cottage cheese category, of 5,88% (*L. monocytogenes*), aspect due to the same extent also to the sheep milk special processing conditions.

Out of the pathogenic species confirmed on the miniApi analyzer, the highest percentage was found in the matured cow cottage cheese matrixes, by 56% for the  $\beta$ -glucuronidase-positive *Escherichia coli*, for the coagulase-positive *Staphylococcus spp.*, of which *Staphylococcus aureus c.p.* – 11,76 %, *Staphylococcus intermedius* – 8,82 % and *Staphylococcus hyicus* – 2,94%;

The detection of the staphylococcal toxin presence, under the research conditions, with the miniVidas analyzer was conducted mainly in the hot season, thus this was highlighted to be present in too low percentages, compared to the dose necessary to the occurrence of the characteristic symptomatology. For the cottage cheese samples, the highest detection percentage

was for the fresh cheese, within 2,00 and 13,20 ng, with a higher quantity in the artisanal processed sheep fresh cheese. (5-13,20 ng).

The incidence of the highlighted *Staphylococcus spp.* strains shows the percentages of the determinations of the strains capable to produce enterotoxin pertained to the *Staphylococcus hyicus* strains with 54,62% for the cow cottage cheese samples and the *Staphylococcus intermedius* strain with 34,52 % for the sheep cottage cheese samples.

For the investigations referring to the assessment of the hygienically condition of the working surfaces correlated to the existence of a quality control system, it was found that the implementation of the H.A.C.C.P. system procedures and monitoring of the same are performed more efficiently in unit A, where the improper tests percentage does not exceed 10,52 %;

By the performance of 1376 tests, the number of somatic cells was assessed, with 567 tests for the industrial processed milk in the Balkan cheese, 356 tests for the artisanal processed milk, 228 raw material milk samples, which have been tested by rapid method of incipient mastitis detection and through 225 comparative analyses for the samples drawn in order to establish the uncertainty degree of the methods used by manufacturers.

The investigations conducted in order to assess the hygiene condition of the working surfaces, of the equipment, tools and packaging in the plants where the milk is industrially processed into Balkan cheese, were performed on a number of 117 tests on working and contact surfaces, 96 microbial aeroflora tests of the technological enclosures, 116 sanitation tests on palmar surfaces of operators, 56 sanitation tests on the operators' working equipment, 118 sanitation tests on the tools and equipment surfaces.

The hygiene assessment of the technological flow in traditional and artisanal system plants was conducted on 115 sanitation tests on working surfaces, tool and palmar surface.

The assessment of the hygiene and quality of the Balkan cheese in addition to the HACCP system implementation was conducted on 936 sanitation tests;

The water used in the Balkan cheese manufacture was investigated by performing microbiological tests for 149 water samples in water supply networks or own water supply, by complying the results achieved with the requirements of STAS 1342/1991, with the working method required by STAS 3001/1991 and 135 water samples, 98 originated in the industrial sources and 39 taken from sheep pens, in order to assess the water hygiene quality by microbiological tests according to SR EN ISO 9308-1 /2004, SR ISO 8199/2005 and ISO 6887-1/2002, and in accordance with law 458/2002 as amended by Law 311/2004 regarding the drinking water.

For 1156 Balkan cheese samples with 352 fresh cow Balkan cheese samples, 292



matured cow Balkan cheese, 300 fresh sheep Balkan cheese and 212 matured sheep Balkan cheese, drawn from the 6 classes of milk processing plants, prevention-oriented tests were performed to monitor the bacterial critical limits.

The Ph.D. dissertation presents comparative aspects of the data achieved through the application of rapid methodology, Quantification Index of *Coliform Bacteria* and of waterborne *intestinal enterococci*, in raw material milk samples.

Another comparative study considered the assessment of the results on incipient mastitis diagnosis, occasion on which the assessment methods of the NCS/ml parameter was determined through parallel use of CMT or R-mastitest, or automated analyzer Porta SCC, of automated analyzer Somascope MKII (SR ISO 13366- 3/2001) by the reference microscopic method of SR ISO 13366- 1/1999.

Moreover, concomitant determinations of the number of coliform bacteria, of the *Escherichia Coli* presumptions confirmation in water samples, by two standardized methods and by the rapid method of **IDEXX** Technology – colorimetric rapid qualitative and quantitative tests - Quanti-tray procedure.

The results achieved throughout this study are similar to the results published by other authors having performed similar research, focusing on milk quality and as raw material for the Balkan cheese processing and who consider the relevance of a hygiene conditions for drawing, storage and transport to the processing plants of the raw material milk and who acknowledge the need to implement a food safety management system, so that the finished products should be marketed to consumers only based on their quality certification, first of all by the manufacturer and secondly, by the public control and coordinating authorities, which are of major importance, just as well.

The dissertation also incorporates own data published in the 10 scientific papers (8 as lead author in collaboration with university professors, 2 only in collaboration with teaching staff, specialized in food microbiology or food products sanitary and veterinary expertise), defended in scientific manifestations organized on the occasion of the symposiums held by the „Ion Ionescu de la Brad” University of Agrarian Sciences and Veterinary Medicine of Iași, “Grigore T. Popa” University of Medicine and Pharmacy of Iași and “Petre Andrei” University of Iași, published in various magazines, elements from the dissertation thesis, master’s degree dissertation, which were highlighted by classical and modern methods, by working microbiological procedures validated within L.S.V.S.J. Iași (Sanitary Veterinary Laboratory of Iasi County), RENAR-certified, according to the reference requirements of standard 17025/2005 - General requirements for the competence to carry out tests and/or calibrations, including

sampling, aspects in connection with the hygiene and quality of cow and sheep raw material milk, with the commercial, nutrition and risk factors, whose „vein” is represented by milk and implicitly the quality of cheese obtained by industrial processing or processing within a traditional, artisanal system.