

ABSTRACT

The research thesis entitled „*The hygienic appreciation of the acid dairy products and some type of cheese by mycological and mycotoxicologic examination*” contains 264 pages and is structured, according to actual legal law, in two main parts: first part „*The actual knowledge stage regarding the contamination with fungi and mycotoxines of the dairy products*” containing 62 pages and representing 26%, and second part „*Personal contributions*” which is extended on 177 pages and representing 74%.

The first part, structured in 4-th chapters, are presented information from scientific literature related to the subject of the thesis, information that were used for the interpretation and comparison of the dates obtained in the second part. This part is illustrated by 1 table and 17 figures selected as being suggestive for the synthesised information. The second part is structured in 6-th chapters and contains the aim and orientation of the researches, the used material and methods, the obtained results and their interpretation, being illustrated by 45 figures and 35 tables. The general conclusions and the bibliography close this part.

The researches made for this PhD thesis have obvious relevance in mycology and mycotoxicology domain because were studied the fungi and mycotoxines contamination dynamics of the dairy products, foods very used in human alimentation especially for children.

These nocive elements influents negatively the quality of life, determining serious illnesses with high morbidity and mortality percentages. The obtained results are sustained by scientifically arguments on which the regulation of the state may be relied with the purpose to improve the population life quality, which is the only viable alternative that assures the prosperity of one nation and a normal demographic dynamic.

The illnesses determined by the mycotoxines, which are substances elaborated in the environment by the toxigen fungi and that may reach inside the human and animal organism, are known under the generic name of mycotoxicosis. Their clinical evolution is serious and most of

the time lethal due to the fact that the mycotoxins affect irreversibly the vital organs and tissues. The mycotoxins cannot be inactivated neither by the antibiotics nor by chemotherapies and their smaller molecular weight does not trigger *in vivo* the antibodies synthesis, thus, the use of biologic products - serum and vaccines – for such illnesses is useless.

The researches proposed and studied in this phd thesis had the purpose to follow and establish the intensity of the mycotic and mycotoxinic contamination phenomenon and by identifying the critical control points may be prevented the contamination of the products with such nocive elements that may endanger the public health.

Taking into consideration these facts, the main objectives of the thesis are as follows:

- Assessment of the fungi contamination burden from acid dairy products and some types of cheese by quantitative mycological examinations.
- Isolation and identification from the taxonomic point of view of the fungi by qualitative mycological examinations.
- Assessment of the mycotoxinic burden of the dairy products by mycotoxicological investigations.
- Identification and quantitative assessment of the M1 aflatoxin from the studied dairy products by ELISA method
- The essay to implement a HACCP system inside the milk processing unities in order to obtain pure dairy products.

The research was done on a 4 years period, from 1 October 2005 until 1 October 2009, on a number of 225 acid dairy products and some cheese types such as: 25 yogurt samples, 25 fruit yogurt samples, 25 pasteurised milk, buttermilk and kefir samples, 25 fresh cow cottage cheese samples, 25 sheep and goat telemea cheese samples, 25 cow and buffalo telemea cheese samples, 25 fresh sheep cheese samples, 25 pressed samples and 25 fungi cheese samples. The researches were made in Mycology and Mycotoxicology laboratory from the Faculty of Veterinary Medicine, Iasi.

After the quantitative mycological examination it was dropped the conclusion that out of all 225 dairy products samples examined by mycological quantitative examination, 135 samples had mycological contamination both with yeasts and fungi, representing a percentage of 60% which means a high percentage. Due to the fact that many of the contaminated products were exceeding by far the maximal legal admitted limits, it may be sustained the idea that these products may be compromised from organoleptical point of view, but they may also have implications on food safety for the consumer.

Concerning the contamination of the acid dairy products (yogurt, fruit yogurt, pasteurised milk, buttermilk and kefir) it may be said that from quantitative point of view these presented a high mycotic burden, the highest fungi microorganism quantity being found on fruits yogurt samples ($6,4 \times 10^5$ CFU/mL).

The contamination of these products happened probably after the pasteurisation process, in the following stages of the technological process, due to the fact that the hygiene conditions were not respected. Another source of contamination for these products may be the lack of sterility of the package, either because it was not respected the proper decontamination time or because the ultraviolet lamp, which is used to sterilize the yogurt recipe, was not working properly. The relatively high contamination percentage of 52% from fruit yogurt samples may also be due to supplementary contamination by adding different sorts of fruit pulp and the multitude of flavours added in the recipe.

The fresh cow cottage cheese were contaminated in percentage of 48%, having a maximal contamination value of 2×10^7 CFU/g. This high quantitative contamination of the samples may be explained because this is a fresh product, that does not contain conserving substances, but also because the hygienically conditions during technological process were not respected, storing at inadequate conditions, disrespecting the conservation thermal parameters but also due to repeated handling.

Due to the fact that the contamination of the samples conserved by pickling was intense, of $1,5 \times 10^6$ CFU/g for sheep and goat telemea cheese and of $5,8 \times 10^7$ CFU/g for cow and buffalo telemea cheese, we may draw the hypothesis of probable existence of two contamination sources: contamination of the conservation solution (of brine) by disrespecting the hygiene conditions. In the case of telemea cheese prepared from pasteurised milk, the high mycotic burden may be explained, on one hand, by secondary contamination of the raw material after pasteurization and, on the other hand, by inadequate marketing conditions (inadequate temperature, repeated handling, lack of hygiene on transportation recipes and of the depositing and commercial spaces).

The massive contamination of the fresh sheep fresh cheese, product that is traditionally manufactured and sold in agro-alimentary markets, may be due to its traditional preparation with unpasteurised milk. Due to the fact that this type of cheese was contaminated in proportion of 100% with a maximum value of $5,5 \times 10^7$ CFU/g, this fact indicates serious deviations from the laws of hygiene on the entire production flux, starting with milking and ending with storing and unhygienic marketing of the product.

The pressed cheese samples and fungi cheese samples were contaminated in proportion of 68% and respectively of 60%, the maximal values for colonies forming units obtained after the mycological examination were of $5,1 \times 10^6$ for pressed cheese samples and of $9,6 \times 10^6$ for fungi cheese samples. The surface of the cheese is considered as a good substrate for the development of fungi, moreover, the majority of these products are matured in open space to a relatively high humidity. Although for these types of cheese both yeasts and fungi play a very important role in maturation process, in majority of cases the development of them must be prevented. Leaving aside the economical loss determined by the alterations of these microorganisms, the presence of fungi might present a considerable interest for human health due to the presence of mycotoxins, toxically fungi metabolites.

Besides the contamination possibilities during maturation process, this significant mycotic contamination might be determined by repeated handling, because the majority of these samples were taken from supermarkets where these products were weighted and wrapped individually out of a bigger piece by the workers of the supermarket.

In conclusion, respecting the sterile hygiene conditions on all production and marketing flux represents the optimal solution that guarantees the quality of the product and the safety of the consumer, even for the products prepared traditionally without the pasteurisation of the raw material, although this practice will be limited or even excluded by the European legislative regulations.

For the identification of the mycomycetes, evidenced by the quantitative mycological examination, and their taxonomic selection is necessary the corroboration of cultural aspects of the colonies, the microscopically morpho-structural particularities the biochemical and genetic features. For this were led a test series with the identification purpose.

After the examination of the mycological burden of acid dairy products and some types of cheese were isolated 319 fungi strains and was determined that the mycological contaminant flora belongs to the following genre: *Candida* (64,1 %), *Penicillium* (32%), *Geotrichum* (14,1%), *Saccharomyces* (5,6%), *Trichosporon* (3,5%), , *Cryptococcus* (1,3%), *Rhodotorula* (0,9%) and *Mucor* (0,6%).

After the quality mycological examination it may be drawn the conclusion that the most frequent isolated strains (64,1%) were those belonging to *Candida* genus, being identified 204 strains represented by the following species: 39 strains of *Candida kefyr*, 35 strains of *Candida sphaerica*, 29 strains of *Candida lipolytica*, 29 strains of *Candida famata*, 14 strains of *Candida intermedia*, 12 strains of *Candida zeylanoides*, 7 strains of *Candida catenulata*, 7 strains of *Candida inconspicua*, 6 strains of *Candida lambica*, 6 strains of *Candida parapsilosis*, 4 strains

of *Candida krusei*, 4 strains of *Candida colliculosa*, 4 strains of *Candida valida*, 3 strains of *Candida holmii*, 3 strains of *Candida norvegensis* and 2 strains of *Candida sake*.

From *Geotrichum* genus were identified 45 strains (14,1%), 35 strains of *Geotrichum candidum* and 10 strains of *Geotrichum capitatum*, from *Penicillium* genus were identified 32 strains, but at species level were identified only those strains used for starter cultures, respectively 14 strains of *Penicillium camemberti* and 13 strains of *Penicillium roqueforti*, the rest of 5 strains being identified only at genus level.

The rest of the genres were identified in a smaller proportion, such as *Saccharomyces* genus was identified in 18 strains (5,6%) with *Saccharomyces cerevisiae* type, out of 11 strains from *Trichosporon* genus (3,4%) were identified 7 strains of *Trichosporon inkin* and 4 strains of *Trichosporon asahii*, 4 strains of *Cryptococcus* with *Cryptococcus curvatus* and *Cryptococcus laurentii* species, 3 strains from *Rhodotorula* genus with two strains from *Rhodotorula mucilaginosa* specie and one *Rhodotorula rubra* specie and two strains of *Mucor* genus.

Through a year of synthetic speech results, it can be said with certainty that mycotoxin analysis performed by competitive ELISA method which was interested in 150 samples of plain yogurt, fruit yogurt, cottage cheese, fresh curd sheep cheese and cheese mold have been a successful experimental model that provides opportunities objective interpretation of a phenomenon with wide and serious epidemiological implications. Of the 150 samples studied, three samples, two representing the cream cheese and a cheese mold, were considered unfit for consumption, aflatoxin M1 content in excess of the maximum allowed by European standards.

Nevertheless, the worst aspect that surprised him and it highlights this study is the large number of mycotoxins contaminated samples and representing 57.3% of total samples analyzed. Even if contamination levels are below their maximum rate of eligibility, however, they provide a clear picture of quality raw materials used to make cheese and yogurt, how their processing and transport conditions, storage and marketing. These dairy products containing aflatoxins should be treated epidemiologically as potentially hazardous factors immunologically vulnerable people, the elderly, sufferers of chronic diseases and especially children.

It should also be taken into account the phenomenon of mycotoxin accumulation in the liver at least, because the enzymatic detoxification of aflatoxin B1 mycotoxin parental form milk AFM1 is a complex biochemical process which takes place stage, involving successive or concurrent a wide range of enzymatic compounds. So detoxification of mycotoxins in the body that the liver does not occur instantaneously and then replenish the body with toxic compounds such nutrients through contaminated with even very small doses, can generate mycotoxicoses concurrently with chronic evolution

The results obtained by a systematic study, which concerned a large variety of dairy products demonstrates the strength of scientific arguments that these animal products may be risk factors for the health of those who consume them, and a stricter control on their content mycotoxins it must be placed by necessity and urgency, even in traditional agro-food markets where these products are marketed by private processors.

The implementation of the HACCP system had as main purpose the assurance of quality and safety of the final product in alimentary industry, especially in dairy industry and it presented a remarkable improvement regarding the safety and the quality of the product. The identification of the CCP in cheese production line determined a satisfactory reduction of the risks, thus leading to the production of less faulty products than in the past. Moreover, the milk producing unity in which the researches took place and that has implemented the HACCP programme, has also succeeded to increase its market share, both locally and internationally, hence confirming the importance of implementing the HACCP system.