

# Researches on Incidence of Fungi in Milk and Dairy Products Rendered Profitable in Craiova Markets

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## Abstract

Fungal contamination was observed in 46 milk samples. Contamination level was 100% for *Candida* and *Rhodotorula* (yeasts). Contamination is attributed to deficiencies in milking dairy cows. In the samples of Telemea cheese, the predominant microflora belonged to the species *Candida* and *Rhodotorula*. In milk and cheese samples was not detected aflatoxin M1.

Many types of cheese are excellent substrates for the growth of fungi. Fungi more important are *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor* and *Trichoderma*. Mycotoxins, produced by certain fungi as toxic metabolic substances can be found in dairy products, either from indirect sources when dairy cows consume feeds with mycotoxin (Aflatoxin B1 and its metabolite that passes into milk - AFM1) or from direct sources as a result of growth intentional or accidental fungi.

Many feed from farm crops are consumed later. In the course of vegetative growth but especially during the flowering period, *Fusarium* fungi may infect grains or other fodder crops and produce fusarium toxins, deoxynivalenol, nivalenol, zearalenone, fumonisins and HT2 and T toxins. Aflatoxins are mainly produced by two species: *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B1 is metabolized by ruminants resulting aflatoxin M1, and this metabolite can be transferred to cows' milk.

**Keywords:** fungi, mycotoxins, toxins, yeasts.

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## 1. Introduction

The overall objective of this paper was to determine the incidence of toxicogenic fungi and their production of mycotoxins in some dairy products rendered profitable in Craiova market [1]. It is based on the idea that vegetables and dairy products form the greatest of the daily human diet during summer. Regular monitoring of the presence of toxigenic fungi and their mycotoxins in milk and dairy products is essential for the establishment of strategies to control and prevent mycotoxicosis in humans and animals [2, 3]. The causative agent, the symptoms, the presence of fungi and their mycotoxins are

necessary to predict the level of risk in the consumption of dairy products on human health or feed use on animal health and the safety of their production (meat and milk). Recently, toxicogenic fungi have got the interest because of their production of toxic secondary metabolites (mycotoxins) in the specific substrates of food and which cause illness in humans and animals [4, 3]. In general, fungal contamination was underestimated by food hygienic-sanitary inspections as the presence of the fungi was not considered to be a risk to public health. Mycotoxins produced by certain fungi as a toxic metabolic substances can found in dairy products from two origins: indirect contamination, which results when dairy cows ingest feed that contain mycotoxins that pass into the milk such as aflatoxin M1, and direct contamination, which occurs because of the international or accidental growth of fungi. In Telemea cheese incidence of

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fungi indicates that the predominant micoflora belong to the genus *Candida* and *Rhodotorula*. Cheese samples collected, did not show detectable levels of aflatoxin M1.

## 2. Materials and methods

In order to accomplish this paper, information, principles and methods were needed to implement complete and effective solutions for diminishing the contamination of mycotoxins with milk and dairy products and implicitly reducing their effects on human and animal health [4, 5].

The study consisted in the mycological examination of 46 samples of raw cow's milk and 72 samples of cow's Telemea cheese, marketed in the markets of Craiova, for the determination of the fungal burden and the predominant genres. Research undertaken and laboratory analyses were conducted at DSVSA and DSP Dolj.

In all Craiova markets there is a wide range of dairy products industrially manufactured but especially crafted by individual dairy cattle farmers [2]. The organoleptic properties of dairy products depend on the characteristics of the milk and its production area. In fact, indigenous microorganisms are responsible for the differences between maturing processes, especially for cheese.

Several mold species (*Aspergillus fumigatus*, *Fusarium oxysporum*, and *Penicillium expansum* are microaerophilic, capable of developing at low oxygen levels as is in the the center of cheese or in the hermetically sealed products. *Aspergillus flavus* is xerophilic (growths on substrates in which water activity is below 0.80) or tolerates salt [6, 7].

Estimation of fungal contamination of milk and

dairy products has a hygienic-sanitary importance. Examination of infected material with fungi was done in the laboratory using direct examination, magnifying examination and stethoscope examination. To facilitate the identification of the microorganism, the materials under analysis were kept in the optimum conditions of temperature and humidity to create to fungus development and fructification conditions.

The material used was firstly washed and disinfected to the surface to remove microflora from the outside, which would develop in abundance, preventing externalization. of the true pathogen that would eventually be found he inside the tissues.

The pathogen material has been put in the Petri dishes, crystallizers and desiccators, on the bottom of which was placed filter paper soaked with sterile water.

The temperature needed for development was ensured by the introduction of the dishes in the thermostat (24-26°C), or left in the laboratory at room temperature (20-22°C). After the development of the mycelium, identification of the fungi was done either directly at the microscope or by isolation on an appropriate medium. Fungi isolated on a nutrient medium are named "isolated". Reference biological material: ATCC fungi strains preserved in glycerol 40%, at - 75°C.

## 3. Results and discussion

The study consisted in the mycological examination of 46 samples of raw milk from cows for determination of fungal burden and predominant genres (Table.1).

**Table 1.** Fungal species isolated from milk and telemea cheese samples

Isolated genre	Number of infected samples			
	Milk	Incidence %	Telemea cheese	Incidence %
<i>Candita</i> spp	46/46	100.0	36/72	50.0
<i>Rhodotorula</i> spp.	46/46	100.0	36/72	50.0
<i>Penicillium</i> spp	19/46	41.3	9/72	12.5
<i>Clodosporium</i> spp	15/46	30.6	6/72	8.3
<i>Aspergillus</i> spp	10/46	21.7	6/72	8.3
<i>Mucor</i> spp	8/46	17.4	4/72	5.5
<i>Rhizopus</i> spp	7/46	15.2	0/72	0

Growing a fungus in food is governed in great part by a series of physical and chemical parameters, and their definition is very important in assessing food stability. In practice, the situation is more complex due to the fact that these factors do not act independently but synergistically.

In the milk samples the *Candida* and *Rhodotorula* species were 100% each, *Penicillium* spp. 41.3%, *Cladosporium* spp 30.6%, *Aspergillus* spp 21.7%, *Mucor* spp 17.4% and *Rhizopus* spp 15.2%. In the Telemea Cheese samples, the *Candida* and *Rhodotorula* species were isolated in 50% each, *Penicillium* 12.5%, *Cladosporium* and *Aspergillus* 8.3% each and *Mucor* 5.5%. *Rhizopus* spp was not present in Telemea Cheese samples.

In the samples of raw milk, seven species of fungi were identified, and in the Telemea Cheese 6 species of fungi. Fresh milk is a liquid with neutral pH, which is more susceptible to bacteriological alteration, and fungi are a rare problem. However, the lack of proper storage and marketable conditions in Craiova markets, favor the development of lactic bacteria, causing the pH of milk to decrease.

Decreasing the pH of milk favors the development of yeast species. The two *Candida* and *Rhodotorula* yeasts isolated in the 100% of the samples examined are very common milk products obtained by lactic fermentation (yoghurt or some cheese).

At Telemea cheese, all these two yeasts predominate in a much lower percentage compared to milk. Inside the block of cheese, the growth of fungi is limited due to lack of oxygen. Under these conditions the *Penicillium* and

*Cladosporium* species are frequently found in the cheese [5].

Analyzing the proportion of different types of fungi in the 46 analyzed milk samples, it was found that 100% of the samples were contaminated with *Candida* spp. and *Rhodotorula* spp., 44% with *Penicillium* spp; 36% with *Cladosporium* spp., 22% with *Aspergillus* spp., 20% with *Mucor* spp. and 16% with *Rhizopus* spp.

According to Regulation (EC) no. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

- for raw milk from cows: germ content at 30°C (per ml) ≤ 100 000 (\*).

(\*) Variable geometric mean for a period of two months, with at least two samplings per month.

In the analyzed milk samples the number of yeasts and molds did not exceed the maximum admissible limit.

Following the quantitative mycological examination, it was found out that all 46 milk samples subjected to the quantitative mycological examination were found to be micotically contaminated with yeast fungi and filamentous fungi. The minimum micotic load (UFC/g) was  $1 \times 10^3$ , and the maximum of  $80 \times 10^3$ . Since the milk is an excellent substrate for the development of most micro-organisms, its pasteurization is a mandatory measure of reducing microbial load and avoiding potential risks to consumers, while providing a salubrious raw material for the production of other dairy products [4].

**Table 2.** Fungi counts isolated from milk and Telemea cheese

Product	Sample number examined	Positive samples		Minimum CFU/g	Maximum CFU/g	Average CFU/g
		No.	%			
Milk	46	46	100	3,0 x 103,0	16,0 x 103,0	9,4 x 103,0
Telemea Cheese	72	36	50	1,0 x 103,0	9,0 x 103,0	5,3 x 103,0

Dairy cattle can produce milk contaminated with AFM1 if they consume feed with AFB1 [4, 6, 7]. Aflatoxin B1 is rapidly absorbed in the digestive tube and metabolized mainly by the enzymes of the liver, transforming it in the AFM1 (a less toxic metabolite than AFB1), after which it is excreted in the milk and urine. The amount of aflatoxin M1 excreted in the milk as a percentage of ingested AFB1 varies between 1 and 2%

depending on the animal, from a day to another, or from one milking to another.

Due to the AFM1 affinity for milk casein it is also present in the dairy products. Milk and dairy products are foods of prime importance for human nutrition and especially for the nutrition of children. For this reason, milk and dairy products must be systematically controlled [3, 4].

#### 4. Conclusions

It is noted that some samples may have a low fungi load and high mycotoxin content, but there is also the possibility that a product has a large number of yeasts and molds/g and a low or even total absence of mycotoxins.

Surveillance of sanitation of food, feed from mycological and micotoxicological point of view is an important way of preventing human and animal disease. The chemical structure of mycotoxins determines how they act and how to choose the detoxification method.

In a feed a mycotoxin is found very rarely. Mixing several feed components may increase the risk of contamination with multiple mycotoxins. Combined use of mycotoxins can lead to interactive toxic effects (synergism, additive, and antagonist).

The impact of mycotoxins on food intended for human consumption is less acknowledged than the effects of additives, pesticides, heavy metals or microbial agents. On the other hand, diseases produced by micotoxins rarely occur in acute form so that their actual action on the human or animal body is less obvious than that of other contaminants.

Mycotoxins can penetrate in the human body through food-cereals, seeds, spices, fruits, beverages, coffee and indirectly through products obtained from contaminated animals - milk, meat, eggs and their by-products.

The high percentage of yeasts especially in *Candida* and *Rhodotorula* isolated from milk and Telemea cheese reveals the need to apply all hygiene measures in milking and milk processing.

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