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Fungal Contamination of Milk and Some Cheese Varieties Retailed in Markets of Menoufia Governorate

[1] Abd Elrahman Elbagoury.[2] Marwa Mohammed Awad.[3] Fatma Hussein

[1] Elbagoury, Abd Elrahman Professor of Milk Hygiene and Control Department, Faculty of Veterinary Medicine .Menuofia University [2]Mohammed, Marwa Awad . Professor of milk Hygiene and Control Department Faculty of Veterinary Medicine shebien Elkome; EL Monofia University [3] Hussein, Fatma Department of Dairy and Dairy Products Health Control, Faculty of Veterinary Medicine, Menoufia University,

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*Correspondence to
Vet.fatma95@gmail.com

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ABSTRACT

The goal of this research was isolation and identification of yeast and moulds some types of cheese, in raw milk. One hundred and twenty samples have been gathered from Menoufia Governorate, Egypt involving; 30 raw milk, 30 processed cheese 30 tallag cheese and 30 Ras cheese. The outcomes stated which, total frequency probabilities of moulds within raw milk, tallaga, processed, and ras cheese were; 24/30 (80%), 12/30 (40%), 25/30 (83.3%) and 28/30 (93.3%) respectively with mean mould counts 2.7×103±1.6×103, 3.4×102±2.6×102, 5.9×103±1.3×103 and 5.4×103±1.6×103 cfu/g, respectively. Penicillium spp. and aspergillus spp. were the mould species that were isolated from these samples. While, the yeast contamination has been identified in Raw milk, processed, Ras and Tallaga cheese have been contaminated with yeast at percent of 66.6, 60, 33.3 and 80% respectively with mean count values of 45.6×102±8.4×102, 7.6×102±2.3×102, 5.6×102±1.2×102 and 13.8×103±3.4×103, respectively. Sacharomyces cervisioe and Candida tropicalis were the most frequently isolated yeast species from the samples. In conclusion, the presence of high frequency of fungi in cheese and raw milk samples could poses potential public health risks and may result in economically detrimental outcomes because of product spoilage consequently, strict hygienic measures are required to be applied through the chain of production of such products.

1. Introduction

Milk and its products are being nutritious food for human and widely regarded as optimal environments for growth of various fungal species, as they contain favorable conditions and essential nutrients necessary for growth of fungi **[1].** The presence of fungi in raw milk is affected by factors such as the physiological condition of the animal, its breeding circumstances, and the environmental conditions throughout storage, milking, and other pre-processing stages Additionally, the sanitary quality of both the cheese and milk was identified by the fungal count. **[2, 3].** Growth of fungi on cheese is a commonly occurred during the ripening process and on surface of hard cheese when it is stored in the refrigerator by both retailers and consumers **[4].** The impact of these microorganisms on the biochemical characteristics of dairy products and milk results in their spoilage, which is characterized by a variety of metabolic by-products. These by-products cause undesirable flavors, odors, and distinct changes in color, texture, and appearance, making them commercially not desired and frequently leading to a decrease in the evaluating of the dairy product. The proliferation of certain yeasts in cheese produced yeasty flavors through the production of alcohol and carbon dioxide, while some yeasts generate sulfides that produce an egg-like odor. Conditions such as the presence of low pH and oxygen levels create an environment conducive to mold growth,

particularly in cheese; however, certain molds can thrive even in low-oxygen conditions **[5].** Identification and characterization of fungi found in cheese and raw milk was carried out by analyzing their phenotypic features, which were determined by their gross cultural and microscopic characteristics, as well as their genotypic characters **[6].** The health of animals and humans can be negatively impacted by certain molds, that are capable of producing mycotoxins, which are fungal 2^{ry} metabolites. These mycotoxins may go in animal and human food chain through either indirect or direct contamination **[7].**

Mycotoxins, particularly aflatoxins might be contaminated milk and its products s either directly by accidental growth of fungi from unhygienic media of processing or the usage of polluted fungal starter cultures that used in manufacturing and then toxins production or indirectly by ingestion of contaminated feed by AFB1 which excreted into milk as AFM1 **[8].**

There are approximately 20 varieties of aflatoxins, which belong to a broad category of toxic compounds. Among these, only four types—AFB1, AFG1, AFB2, and AFG2—are known to cause natural contamination of food products. Additionally, AFM1 can be present in milk and its derivatives as hydroxyl metabolites, resulting from the consumption of feed contaminated with these toxins by dairy animals **[10].**

Therefore, objective of this investigation was to detect and isolate fungal contamination within some types of cheese and raw milk samples.

2. Material and methods:

2.1. Collection of samples:

A total of 100 and twenty samples of raw milk, processed, Tallaga, and Ras cheese have been gathered from supermarkets and dairy shops in the Menoufia Governorate. The samples have been transported to the laboratory in sterile, airtight jars with minimal delay to be exposed and prepared to a mycological investigation.

2.2. Preparation of cheese samples:

Ten grams of each cheese sample and ten milliliters of raw milk were homogenized in a stomacher bag (Lab-blender 400, Seward, UAC House Friars Road, London SE19UG. Model No. 6021) with ninety milliliters of sterile 0.2 percent sodium citrate solution. To achieve a dilution of 102, one milliliter of the original sample homogenate was added to a test tube that contained nine milliliters of 0.1% sterile peptone water. The same procedure was followed for preparing a ten-fold serial dilution **[11]**.

2.3. Enumeration, Isolation and determination of fungi:

The characterization of different yeast and mould colonies was applied by microscopical examination according to **[12, 13].**

2.4 *Statistical analysis:*

The SPSS program was utilized to conduct a statistical analysis. The outcomes were presented as the mean of 3 readings with the standard error, regarding **[14**].

3. Results:

Table (1): Statistical analytical results of molds count of examined samples (Total mold count per gram). Number=30

Samples Types	Total number of studied samples	Positive sample number	%	Mean ± SE (colony-forming units per milliliter or gram)
Raw milk	30	24	80	$2.7 \times 10^3 \pm 1.6 \times 10^3$
Tallaga cheese	30	12	40	$3.4 \times 10^2 \pm 2.6 \times 10^2$
Processed cheese	30	25	83.3	$5.9 \times 10^3 \pm 1.3 \times 10^3$
Ras cheese	30	28	93.3	$5.4 \times 10^3 \pm 1.6 \times 10^3$

Table (2): Incidence and statistical analytical outcomes of total yeast count per gram for examined samples (Number=30)

Samples Types	Total number of studied samples	Positive sample number	%	Mean ± SE (colony-forming units per milliliter or gram)
Raw milk	30	20	66.6	$45.6\times10^2\pm8.4\times10^2$
Tallaga cheese	30	18	60	$7.6\times10^2\pm2.3\times10^2$
Processed cheese	30	10	33.3	$5.6\times10^2\pm1.2\times10^2$
Ras cheese	30	24	80	$13.8\times10^3\pm3.4\times10^3$

Table (3) Frequency of fungal isolates in the examined samples (Number =30)

Type of moulds	Raw milk		Tallaga cheese		Processed cheese		Ras cheese	
	No.	%	No.	%	No.	%	No.	%
Aspergillus flavus	8	26.6	5	16.6	4	13.3	15	50
Aspergillus niger	2	6.6	------	-------	1	3.3	4	13.3
Aspergillus fumigatus	------	-----	2	6.6	2	6.6	1	3.3
Geotrichum candidum	3	10	------	-------	-----	------	1	3.3
Mucor spp	------	-----	-------	-------	-----	-----	2	6.6
*Penicillium chrysogen*um	2	6.6	-------	-------	------	------	1	3.3
Penicillium hemtrachum	-------	-----	2	6.6	4	13.3	-----	------
Penicillium caseifulvum	2	6.6	-------	-------	2	6.6	1	3.3
Type of yeast								
Candida albicans	------	-----	2	6.6	-----	------	4	13.3
Rhodotorula spp.	5	16.6	-----	-----	-----	-----	-----	-----
Sacharomyces cervisioe	2	6.6	1	3.3	2	6.6	3	10
Candida tropicalis	2	6.6	1	3.3	1	3.3	1	3.3

Table (4): Quality of studied samples regarding *EOSQC (2005)*

Examined samples	Standards according to *EOSQC (2005)*							
	Moulds ≤ 10 cfu/g				Yeast ≤ 400 colony-forming units per gram			
	Acceptable		Unacceptable		Acceptable		Unacceptable	
	No./30	%	No./30	%	No./30	%	No./30	%
Raw milk	6	20	24	80	10	33.4	20	66.6
Tallaga cheese	18	60	12	40	12	40	18	60
Processed cheese	5	16.7	25	83.3	20	66.7	10	33.3
Ras cheese	2	6.7	28	93.3	6	20	24	80

4. Discussion:

The contamination of cheese and raw milk by fungi is a consequence of the unhygienic conditions that happen throughout their manufacturing, transportation, production, and storage periods. Additionally, fungal contamination can be the consequence of contaminated air, unhygienic measures in certain factories, or storage. **[15]**. The provision of all essential nutrients and conditions for development of fungi is the reason why cheese and raw milk are generally regarded as a perfect growth medium for numerous fungal species **[16].**

Within the present research a total of 120 samples (30 raw milk, 30 processed cheese, 30 tallaga cheese, and 30 ras cheese) obtained from Menoufia Governorate, Egypt. Have been analyzed mycological. The outcomes showed in **Table (1)** discovered which, total frequency rate of the mould in tallaga, raw milk, processed and ras cheese samples were; 24/30 (80%), 12/30 (40%), 25/30 (83.3 %) and 28/30 (93.3%) respectively with mean mould counts $2.7\times10^3\pm1.6\times10^3$, $3.4\times10^2\pm2.6\times10^2$, $5.9\times10^3\pm1.3\times10^3$ and $5.4\times10^3\pm1.6\times10^3$ cfu/g. An insignificant variance in the mean mold counts between processed and ras cheeses.

Mold contamination in dairy products serves as an indicator of sanitary quality conditions **[17].** The distribution of isolated mold species from the positive samples is shown in **Table (2**)

In raw milk *Aspergillus flavus* is the most isolate (8/30, 26.6%), *Geotrichum candidum* (3/30, 10%) and Penicillium spp. (4/30, 10.2%) while In Tallaga cheese, the most prevalent isolates were *Aspergillus flavus* (5/30, 16.6%), *Aspergillus fumigatus* 2/30, 6.6%), and *Penicillium hemtrachum* (2/30, 6.6%). In processed cheese, the most common mold isolates were Penicillium spp. (6/30, 19.9%) and Aspergillus spp. (7/30, 23.2%). In ras cheese, the most abundant isolates were Aspergillus spp. (20/30, 66.6%), Penicillium spp. (1/30, 3.3%), *Mucor spp* (2/30, 6.6 %) and *Geotrichum candidum* (1/30, 3.3%). Similar findings were reported by **[18],** he identified Aspergillus spp., Geotrichum spp., Penicillium spp., and Fusarium spp. as the primary fungi responsible for mycotoxin contamination in cheese and milk. **[19]** found that the most prevalent genera among the four types of cheese samples examined were Aspergillus and Penicillium species. Additionally, **[20]** noted that Penicillium was the most common mold genus found in cheese samples, after that Aspergillus, Geotrichum, Cladosporium, and Mucor species.

The outcomes documented within **Table (3)** was noted that the studied samples of Tallaga, Raw milk, Ras and processed cheese have been contaminated with yeast at percentage of 60, 66.6, 80 and 33.3% respectively with mean count values of $7.6\times10^{2}\pm2.3\times10^{2}$, $45.6\times10^{2}\pm8.4\times10^{2}$, $13.8\times10^{3}\pm3.4\times10^{3}$, and $5.6\times10^{2}\pm1.2\times10^{2}$ respectively. **[21]** noticed a nearly identical frequency of yeast in Tallaga cheese samples. They analyzed fifty soft white cheese samples mycologically and determined which frequency of yeasts was sixty. **[22] and [23]** discovered greater outcomes, whereas **[24] and [25]** noticed fewer frequency and counts. **[26] and [27]** reported a relatively fewer frequency and counts in processed cheese. However, **[28]** achieved a more favorable result. **[29]** reported fewer frequency and counts of Ras cheese, while **[30] and [19]** reported a greater frequency and counts. Yeasts and mold count in cheese and raw milk are utilized as an indicator of the quality of sanitation. These counts could be the outcome of contaminated air, a change in season, a poor environment in certain factories, unregulated sterilization, the utilization of milk of inferior quality, equipment, and storage, unsensitized instruments, and other factors.

The most predominant yeast species as shown in **Table 2** were isolated from raw milk were *Rhodotorula* 5 (16.6%), *Sacharomyces* 2 (6.6%) and *Candida tropicalis* 2 (6.6%). While in Tallaga cheese were *Candida albicans* 2 (6.6%) *Sacharomyces* 1(3.3%) and *Candida tropicalis* 1 (3.3%). In processed cheese only *Sacharomyces* 2(6.6%) and *Candida tropicalis* 1 (3.3%). **[31]** achieved outcomes that were nearly identical. In the processed cheese samples that were investigated. *Candida albicans* is the most type of yeast in Ras cheese 4(13.3%), followed by *Sacharomyces* 3 (10%) and *Candida tropicalis* 1 (3.3%) lower than result recorded by **[32].**

Regarding the outcomes in **Table (4),** 80, 83.3, 40, and 93.3% of the studied raw milk, processed, Tallaga, and Ras cheese samples, respectively had molds more than the acceptable limit (undesirable) and in comparison, to EOSQC (2005), the yeast counts were 66.6, 60, 33.3, and 80 percent higher. Such numbers are considered undesirable. Certain food borne yeasts & molds may be hazardous due to their potential to allergic reactions **[33].** Additionally, the proliferation of fungi on cheese can lead to discoloration and undesirable flavors, as well as the production of mycotoxins, which have been linked to numerous instances of mycotoxicosis **[34].**

Conclusion

This study has clearly demonstrated the presence of fungal contamination in cheese samples and raw milk obtained from markets in the Menoufia Governorate, Egypt. This finding suggests inadequate hygienic practices during the processes of milk production, cheese & handling manufacturing, storage and ripening process. The predominant mold species identified have been Penicillium and Aspergillus spp. The proliferation of these fungi poses potential health risks to customers. Consequently, it is imperative to implement stringent hygienic measures throughout production of milk, cheese processing, handling, and storage, ensuring that final products adhere to Egyptian standar.

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