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Mycological Evaluation of Egyptian Ras Cheese with Special Reference to Mycotoxins

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ABSTRACT

Egyptian Ras cheese (Roumy cheese) is a popular cheese due to its affordable price and considered as one of the most harmful cheeses for human health, and we are not even referring to its high content of saturated fats and not to its high salt content but to the contamination with different types of mycobiota and its harmful toxins. The purpose of this study is to enumerate, isolate and identify mycobiota contaminating such cheeses. In addition, determination of mycotoxin residues in the examined Ras cheese samples and compare their incidence in the surface (Rind) and core in the examined cheese samples after cutting 2.5 cm from the rind. Therefore, One hundred Ras cheese samples were collected from supermarkets, small diaries, groceries in Alexandria governorate, Egypt. The obtained results revealed that the examined Ras cheese samples were contaminated with yeast with a mean count of $7.5\times 10^6 \pm 1.1\times 10^6$ cfu/g and $5.8\times 10^4 \pm 1.04\times 10^4$ cfu/g for surface and core with an incidence of 54% and 70%, respectively. While, the incidence of mould contamination were 59% and 56% with a mean count of $4.9 \times 10^5 \pm 0.83 \times 10^5$ cfu/g and $6.33 \times 10^3 \pm 1.9 \times 10^3$ cfu/g for surface and core, respectively. Aflatoxins M1, M2, B1, B2, G1, G2 and Ochratoxin A were detected in the surface of examined pooled Ras cheese samples with an incidence rate of 41.66, 25, 33.3, 25, 16.6, 8.3 and 16.6%, respectively. Core of examined pooled Ras cheese samples were also contaminated with the mentioned aflatoxins types except AFM2 and AFG2 were not detected while the determined mycotoxins types were AFM1, AFB1, AB2, AG1 with an incidence of 16.6, 33.3, 16.6 and16.6%, respectively. Ochratoxin A has been detected only in group number 9 of pooled Ras cheese samples with and incidence of 8.33%. By all accounts, and with proven results; it is obvious that the majority of collected ras cheese samples were contaminated with a wide range of mycobiota and their secondary metabolic toxins "mycotoxins". Strict hygienic measures should be applied to plants of cheese production in order to reduce the risk of mould growth and mycotoxins release.

1. INTRODUCTION

Egyptian Ras cheese (Roumy cheese), also known as gebna torki in Alexandria governorate and it is considered as one of the main traditional hard cheeses in Egypt. It has a pungent smell, and different degrees of saltiness depending on the age. It is thought to be derived from the Greek kefalotyri cheese (Dabiza and El-Deib, 2007; Hatem et al., 2012).

Roumy cheese is made from raw cows' milk, or from a mixture of cow's and buffalo's milk without using starter culture. Peppercorns may be added to enhance the taste. After 3–6 months the cheese develops an open texture and a sharp, pungent flavor. The main problem occurs in the storage areas where the cheese is placed for months to develop its texture during ripening period which are supposed to be cleaned daily, but unfortunately they don't get cleaned at all. When the Roumy cheese is brining or sprinkled by salt and pressured by molds the salted water drips onto the wood shelves where the cheese blocks sit for months, leaving the wood to absorb and build up diversity of microbial contamination including bacteria and fungi. The rotting fungi get transformed into the cheese blocks and starting producing different types of toxins called mycotoxins which include aflatoxins. Aflatoxin is an odorless and colorless toxin produced by certain molds that can cause liver damage and cancer. Before the cheese is

sent out to supermarkets, it became cleaned with a special brush trying to remove the mouldy rind or surface to render it acceptable by consumers, however, it only cleans about 1 to 2mm but by then the toxins have already reached many centimeters into the cheese disc by the end of the ripening period (Hofi et al., 1970).

Cheese ripening is a dynamic and complex biochemical process that includes fat hydrolysis, protein breakdown and lactose metabolism. These processes are catalyzed by many agents such as indigenous milk enzymes, residual coagulant, starter or non- starter microflora and secondary microrganisms including moulds. Presence of moulds on the surface of mould-ripened cheese gives them a different appearance and flavor from other cheeses (Hayaloglu and Kirbag, 2007). Moulds have a more complex enzyme system than bacteria and their enzymes contribute to the maturation of the cheese, i.e., to proteolysis and lipolysis which are more extensive in these cheeses (Gripon, 1987).

Hard and ripened cheeses are better preserved due to the lower pH, lower water activities (aw) and salinity (Ledenbach and Marshall 2010). If the curd mass is unevenly distributed and insufficiently pressed, it will increase the risk of cheese spoilage (Sheelan, 2007).

Mycobiota are significant spoilage microorganisms of foodstuffs during the storage period and rendering them unfit for human consumption by retarding their nutritive value and sometimes by releasing mycotoxins. Fungal growth on cheese is a common problem for the cheese manufacture during ripening as well as for the retailer and consumer during refrigeration storage period (El-Fadaly et al., 2015).

Mycotoxins are the secondary metabolites for mycobiota reported to be potentially harmful to animals or humans. Predominant species with aflatoxin production ability include A. flavus and A. parasiticus (Yu et al.2004). Mycotoxins can be present in dairy products from two origins: (a) indirect contamination, which results when dairy cows ingest feed that contains mycotoxins that pass into the milk such as aflatoxin M1, and (b) direct contamination, which occurs because of the intentional or accidental growth of molds. They are naturally occurring molecules and are thought to confer a selective advantage to the producer strain within complex ecosystems (Seddek et al., 2016).

The aim of this study is to evaluate mycologically the examined Egyptian Ras cheese through enumeration, isolation and identification of moulds with special reference to mycotoxins production by moulds.

2. MATERIALS AND METHODS

2.1. Collection of samples:

A total of one hundred samples of Egyptian Ras cheese were collected randomly from supermarkets, groceries and small dairies at Alexandria Governorate. The samples were obtained as sold to the public and transferred as soon as possible in an icebox at 4 ± 1 ◦C to the laboratory with a minimum of delay to be examined mycologically.

2.2. Preparation of Ras cheese samples:

Each sample was about 250 grams, transferred aseptically in a sterile polyethylene bag. The collected samples were divided into two groups Surface (rind) and core by using sterilized knife at a distance of one inch from the edge of the surface to make a mycological comparison between surface and core. Each sample was cut into very small pieces (milling), then thoroughly mixed.

2.3. Mycological evaluation of examined Ras cheese samples:

2.3.1. Preparation of 10-folds serial dilutions using sodium citrate with 2% dilution according to (APHA, 1985).

2.3.2. Yeast and mould counts according to (Bailey and Scott, 1998).

2.3.3. Identification of isolated moulds:

The isolated mould strains on Sabaroud dextrose slope were sub cultured on the Sabaroud dextrose plates by three point inoculation technique, and incubated for 3 – 5 days at 25 ◦C then identified macroscopically according to Samson et al. (1995).

3.5. Determination of Mycotoxin residues in examined Ras cheese samples: by Extraction and clean-up procedures for high-performance liquid chromatography (HPLC) analysis. The HPLC was an Agilent 1100 HPLC system, Agilen technologies, Waldbornn, Germany (Galavano et al, 1998)

3. RESULTS AND DISCUSSION

3.1. Mycological evaluation of examined Ras cheese samples:

Yeasts tend to grow within drink and food matrices in a planktonic form and they tend to ferment sugars, growing well under anaerobic conditions. Molds, on the other hand, tend to grow on the surface of objects in the shape of a visible mycelium‟ made up of many cells. Both yeasts and molds cause various degrees of deterioration and decomposition of foods (FDA, 2017). Strong yeast growth on cheese can lead to defects in aroma and flavor (yeasty, moldy, putrid, overripe, alcoholic,

musty, fermented, earthy, spicy, ammonia, pungent, rancid, sweet, and gassy) (Hayaloglu, 2016). Mould contamination of cheese not only lead to spoilage and cause sensory and economical losses but also lead to production of mycotoxins causing potential health hazards to human due to their teratogenic, mutagenic, carcinogenic effects leading to hepatocarcinoma and organ damage (Darwish et al., 2014).

3.1.1. Yeasts in examined Ras cheese samples:

Results given in Table (1) illustrated that 54% and 70% of surface and core of examined Ras cheese samples were contaminated with yeast with a mean count of $7.5\times 10^6 \pm 1.1\times 10^6$ and $5.8\times 10^4 \pm 1.04\times 10^4$ for surface and core, respectively.

According to ES (1007-5/2005): which stipulated that Ras cheese should not contain more than 100 cfu/g of yeast. Therefore, all positive samples were not complied with the established limit. The relative high concentration of yeast count in examined samples may be due to the ability of yeast to tolerate high acidity (Battcock and Azam-ali 1998).

A health risk from yeasts exists when facultative pathogens appear in food or on food-contaminated equipment. Facultative pathogens are known to cause infections in susceptible individuals such as infants, seniors, immunocompromised persons, persons with AIDS, diabetics, alcoholics, and pregnant women. In young children, oral thrush and nappy rash are not unheard of; allergies can also be involved. Immunosuppressed individuals can suffer from a serious mycosis of the organs. More than 50% of all fungal infections are caused by *Candida* spp. Opportunistic pathogenic yeasts usually found in milk from mastitic cows (Fuquay et al., 2011 and Hidalgo, 2011).

3.1.2. Mould in examined Ras cheese samples:

Results given in **Table (2)** illustrated that 59% and 56% of examined surface and core of collected Ras cheese samples were contaminated with moulds ranging between 2.0×10^4 to 3.0×10^6 cfu/g with a mean count of $4.9 \times 10^5 \pm 0.83 \times 10^5$ cfu/g and 1.0×10^3 to 1.03×10^5 cfu/g with a mean count of $6.33 \times 10^3 \pm 1.9 \times 10^3$ cfu/g for surface and core, respectively.

According to Es: (1007-5/2005) which stipulated that Ras cheese should not contain more than 10cfu/g of mould. Therefore, all positive samples were not complied with established limit for mould.

Table (1): Statistical analytical results of yeast count (cfu/g) of examined Ras cheese samples: (n=100)

cuts	Positive samples		Minimum	Maximum	Mean ± SE
	No.	%			
Surface	54	54	1.0×10^5	3.04×10^7	$7.5\times 10^6 \pm 1.1\times 10^6$
Core	70	70	1.0×10^3	3.64×10^5	$5.8\times 10^4 \pm 1.04\times 10^4$

Table (2): Statistical analytical results of of mould count (cfu/g) of examined Ras cheese samples: (n=100)

cuts	Positive samples		Minimum	Maximum	Mean ± SE
	No.	%			
Surface	59	59	2.0×10^4	3.0×10^6	$4.9 \times 10^5 \pm 0.83 \times 10^5$
Core	56	56	1.0×10^3	1.03×10^5	$6.33 \times 10^3 \pm 1.9 \times 10^3$

Table (3): Frequency distribution of moulds isolated from the examined Ras cheese samples.

Mold isolate	**Surface**		**Core**	
	No	**%**	**No**	**%**
Absidia cormbifera	0	0	2	1.25
Acremoium spp.	1	0.6	0	0
Alternaria alternata	7	4.29	5	3.12
Aspergillus spp.				
A.niger	27	16.5	26	16.25
A. flavus	13	7.97	17	10.6
A. fumigatus	0	0	5	3.12
A. ochraceus	6	3.68	1	0.62
A. candidus	5	3.06	1	0.62
A. terreus	1	0.6	0	0
A. nodulans	1	0.6	0	0
Chatmeom spp.	3	1.84	0	0
Cladosporium	30	18.4	24	15
Geotricum candidum	1	0.6	2	1.25
Mucor spp	9	5.5	15	9.37
Paeciliomyces	2	1.22	2	1.25
Penicillium spp	53	32.5	58	36.25
Rhizopus spp	4	2.45	2	1.25
Total	163	100	160	100

Table (4) Incidence and levels of *Aflatoxin* (M1 and M2) and Ochratoxin A (ppb) in the examined Ras cheese sample by HPLC

Product	**No. of pooled groups**	**Levels Mycotoxins (ppb)**								
		AFM1			**AFM2**			**Ochratoxin A**		
		No	**%**	**Range**	**No**	**%**	**Range**	**No**	**%**	**Range**
Surface	12	5	41.66	0.197-2.355	3	25	0.284-1.007	2	16.6	0.693-1.508
Core	12	2	16.6	0.171-0.309	ND	ND	ND	1	8.33	0.118

Table (5) Incidence and levels of *aflatoxin* (B1, B2, G1 and G2) (ppb) in the examined Ras cheese sample by HPLC

Product	**No. of pooled groups**	**Levels Aflatoxins (ppb)**											
		B1			**B2**			**G1**			**G2**		
		No	**%**	**Range**	**No**	**%**	**Range**	**No**	**%**	**Range**	**No**	**%**	**Range**
Surface	**12**	4	33.3	0.047-0.926	3	25	0.060 - 0.542	2	16.6	0.135 – 0.289	1	8.3	0.071
Core	**12**	3	33.3	0.218 - 0.767	2	16.6	0.149-0.325	2	16.6	0.066 - 0.110	ND	ND	ND

The lower mould count in the core cheese samples than the surface may be attributed to the one inch cut of the contaminated surface. Also, the fact that mould generally cannot penetrate deep into the hard cheese product and trimming off at least one inch around and below the mould spot will not cross contaminate the other parts (USDA, 2013).

Results in Table (3) showed that the frequency of distribution of mould isolated from surface and core of examined Ras cheese samples were *Absidia cormbifera* 0 (0%) and 2 (1.25%), *Acremoium spp.* 1 (0.6%) and 0 (0%), *Alternaria alternate spp.* 7(4.29%) and 5 (3.12%), *Aspergilus niger* 27 (16.5%) and 26 (16.25%), *A. flavus* 13 (7.97%) and 17 (10.6%), *A. fumigatus* 0 (0%) and 5 (3.12%), *A. ochraceus* 6 (3.68%) and 1 (0.62%), *A. candidus* 5 (3.06) and 1 (0.62%), *A. terreus* 1 (0.6%) and 0 (0%), *A. nodulans* 1 (0.6%) and 0 (0%), *Chatmeom spp*. 3 (1.84%) and 0 (0%), *Cladosporium* 30 (18.4%) and 24 (15%), *Geotricum candidum* 1 (0.6 2%) and 2 (1.25%), *Mucor spp* 9 (5.5%) and 15 (9.37%), *Paeciliomyces* 2 (1.22%) and 2 (1.25%), *Penicillium spp* 53 (32.5%) and 58 (36.25%), *Rhizopus spp* 4 (2.45%) and 2 (1.25%), respectively.

The high incidence percent and frequent isolation of both *Penicillium* and *Aspergillus* species from milk and dairy products may be attributed to their ability to grow over a water activity value ranges from 0.62 to 0.995; over a wide range of pH from 2 to 11; over a temperature ranges from -10 to around 60 °C and over a wide range of nutrient limitations (Pitt and Hocking 2009).

3.2. Determination of mycotoxins residues in the examined Ras cheese samples:

Data presented in Table (4) and Table (5) illuminated that 41.66% of surface of pooled Ras cheese samples were contaminated by mycotoxins. While, only 33% of core of pooled Ras cheese samples were contaminated.

The obtained results showed that Aflatoxins M1, M2, B1, B2, G1, G2 and Ochratoxin A were detected in the surface of examined pooled Ras cheese samples with a range of 0.197 to 2.355 ppb, 0.284 to 1.007 ppb, 0.047 to 0.926 ppb, 0.060 to 0.542 ppb, 0.135 to 0.289ppb, 0.0 to 0.071 ppb and 0.693 to 1.508 ppb with an incidence of 41.66 %, 25%, 33.3%, 25%, 16.6 %, 8.3 % and 16.6%, respectively. Core of examined pooled Ras cheese samples were also contaminated with the mentioned aflatoxins types except AFM2 and AFG2 were not detected while the determined mycotoxins types were AFM1, AFB1, AB2, AG1 ranging between 0.171 to 0.309 ppb, 0.218 to 0.767, 0.149 to 0.325 and 0.066 to 0.110 ppb with an incidence of 16.6%, 33.3%, 16.6% and16.6%, respectively. Ochratoxin A has been detected only in group number 9 of pooled Ras cheese samples with and incidence of 8.33%.

The high incidence of AFM1 is attributed to the high affinity of AFM1 to casein of milk, therefor cheese could be the most potent source of aflatoxin among dairy products (Bakirci, 2001). The increased concentration of AFM1 refers to the presence of high concentration of the AFB1 in the dairy animals feed as AFB1 is converted into AFM1 by enzymes mainly present in the anima's liver and then passed into urine and milk. Many studies have reported AFM1 in various cheeses (Finoli et al., 1983; Taniwaki and van Dender 1992; Gűrses et al., 2004; Kokkonen et al., 2005).

4. CONCLUSION

It is obvious that the majority of Ras cheese samples which have been collected from supermarkets, small diaries and hypermarkets in Alexandria governorates were contaminated with a wide range of mycobiota either yeast and/ or moulds and their secondary metabolic toxins " mycotoxins" rendering cheese a possible source for human health hazards. Contaminated samples give a clear indication about poor sanitary measures starting from feeding of animals with contaminated feed with mould and mycotoxins which lead to contaminated milk used in cheese processing and passing through bad hygienic practices during manufacturing, handling, storage and distribution of Ras cheese samples. Strict hygienic measures should be applied in dairy farms to obtain mycotoxin free milk also application of HACCP system in the plant of cheese production to guarantee the production of safe cheeses free from mould and their toxins.

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