



Mould contamination of some Egyptian cheese

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ABSTRACT

Mould contamination of milk products is a matter of significance in the field of food safety due to its related food spoilage and production of mycotoxins. This study was undertaken to investigate the incidence of mould contamination in different cheese types marketed in Zagazig city, Egypt. Roumy cheese had the highest total mould count followed by cheddar and white cheese types. The prevalent mould genera were *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria*. Further identification of *Aspergillus* (A) species revealed that *A. niger*, *A. flavus*, *A. parasiticus* and *A. ochraceous* are the dominant Aspergilli. The public health importance of the prevalent mould genera was discussed.

Key words: Mould, cheese, health hazards

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1. INTRODUCTION

Cheese is widely recognized as one of the important sources of a vast array of nutrients including protein, fat, vitamins as vitamin D and minerals as calcium, magnesium, and potassium. Scientific studies provide evidence that intake of milk and milk products as cheese is related to improved bone health and lower risk of cardiovascular disease, hypertension, and type 2 of diabetes (Ranganathan et al., 2005). Moulds in some cheese types can cause both economic and sensory problems. Moulds usually present in raw milk do not survive pasteurization; their presence in pasteurized milk and other milk products is caused mainly by re-infection during manufacturing (Jodral et al., 1993). The contamination of milk products, particularly cheeses is caused by moulds present in the environment of cheese factories, like walls and shelves of ripening rooms, air, equipment, water, milk, brine, etc. (Jay, 1992). Mould contamination of dairy products is of a particular importance in the field of food industry. Many factors control the factors affecting the mould growth such as moisture, pH, oxygen, substrate and the interaction with other microbiological agents. Generally, moulds can grow over a wide range of pH, temperature and water activity (a_w) (Pitt and Hocking, 2009). The incidence of fungal contamination of cheese contamination was investigated in different localities of the world such as Spain, Portugal and Slovenia (Tornadijo et al., 1998; Pereira-Dias, et al., 2000; Gotic Torkar and

Vengus, 2008). Despite of the large-scale consumption of cheese by Egyptian population, few reports are available about the incidence of mould contamination in different cheese types distributed in the Egyptian food market. Mould contamination of cheese may lead to spoilage and production of mycotoxins with potential health hazards to human due to their carcinogenic effects, liver diseases and organ damage (Darwish et al., 2014). Thus, this study was undertaken to estimate the incidence of different mould genera in three cheese types commonly consumed by Egyptian population. The examined cheese types included roumy, cheddar and white cheese. The public health importance of the prevalent mould genera was also discussed.

2. MATERIALS AND METHODS

2.1. Collection of cheese Samples

Seventy-five random samples of three cheese types namely, roumy, cheddar and white cheese (twenty-five of each) were collected from different supermarkets in Zagazig city, Egypt. Samples were identified, packed and transferred to the laboratory in an icebox and subjected to the mycological examination.

2.2. Preparation of samples

Twenty-five grams from each cheese sample were aseptically homogenized in 225 ml of sterile buffered peptone water 0.1% at 2500 rpm for 2 min

with 225 ml of 0.1% sterile peptone water using a sterile homogenizer (type M-P3-302, mechanic, precyzina, Poland). Such homogenate represents the dilution of 10^{-1} , and then decimal dilutions were done (APHA 2001).

2.3. Determination of the total mould count

The total mould counts (TMC) were determined by culturing duplicate plates on each of malt extract agar media and Czapecz-Dox agar with 5% NaCl (Oxoid, Basingstoke, UK) followed by incubation in dark at 25 °C for 5-7 days. During the incubation time, the plates were examined daily for the star-shape mould growth, which is picked up under aseptic conditions with its surrounding cultivated medium and transferred into malt extract slope agar (Oxoid) then kept for further examination. Estimation of TMC was obtained by counting of the cultured agar plates of acidified malt extract agar and osmophilic moulds on Czapecz-Dox agar (APHA 2001).

2.4. Identification of isolated moulds

The identification of isolated mould genera were carried out based on their micro morphological properties (Pitt and Hocking, 2009). In brief, the isolates were sub-cultured on malt extract agar and Czapecz-Dox agar, incubated at 25 °C for 5-7 days. The identification of the colonies was carried out by careful observation and measurements of the macroscopic and microscopic characteristics of the mould colonies, which were recorded in data sheet.

2.5. Macroscopical examination

The cultures were examined daily for the rate and pattern of growth during the incubation period. Observations were made for the consistency of the surface growth; the pattern of folding (rugae); the distinctness of the colony margin and for the presence of pigment either on the surface or the reverse of the colony or diffusing into the surrounding medium. Both the surface and backside of the colony were examined.

2.6. Microscopical examination

From the periphery of 5-7 days old mould colony, a triangular piece was transferred to a clean glass slide. With two mycological needles, the piece of the colony was distributed with one or two drops of 70 % alcohol. One drop of lactophenol stain was added after evaporation of the alcohol. Then the slide was covered by a clean cover slide followed by gentle pressure to remove the excess of fluid and air bubbles as well as to depress the hyphae and other structures for facilitating microscopic examination. The prepared slides were

examined under low power and oil immersion lens to characterize the measurements and morphological structures of the mould growth, concerning the conidial stage, head, vesicle, sterigmata, conidiophore and conidia.

2.7. Evaluation of lipolytic and proteolytic activities of the existed moulds

Effect of lipase activity on Tween 80 was done (Kotula et al., 1982) and on tribytrin (Alford 1976). Proteolytic activity was investigated using opaque skim milk agar medium to detect and quantify the magnitude of proteolysis (Harrigan et al., 1966).

2.8. Statistical analysis

All values are expressed as means \pm SD, and all measurements were carried out in duplicates. Mould counts were converted into base-10 logarithms of colony forming units per g of meat product samples (\log_{10} CFU/g). Statistical significance was evaluated using the Tukey-Kramer HSD test (JMP statistical package; SAS Institute Inc., Cary, NC). In all analyses, $P < 0.05$ was taken to indicate statistical significance.

3. RESULTS

The obtained results shown in (Fig. 1) revealed that roumy cheese had significantly higher TMC followed by cheddar and white cheese. The recorded mean \pm SD (\log_{10} cfu/g) values were 3.61 ± 0.18 , 3.17 ± 0.12 and 2.69 ± 0.14 in the examined roumy, cheddar and white cheese, respectively. As similarly, roumy cheese had the highest incidence percentage 80% (20/25 positive samples), followed by cheddar cheese 64% (16/25) and finally white cheese 52% (13/25) respectively, as declared in (Fig. 2).

Aspergillus, *Penicillium*, *Cladosporium* and *Alternaria* could be isolated with different percentages from the examined samples as shown in (Fig. 3). *Aspergillus* could be isolated from the examined roumy, cheddar and white cheese with percentages of 88%, 60% and 20%, respectively, while in case of *Penicillium*; these values were 80%, 48% and 20%, respectively. *Cladosporium* was isolated from roumy, cheddar and white cheese with percentages of 40%, 28% and 20%, respectively, while in case of *Alternaria*; these values were 20%, 20% and 8%, respectively.

The different *Aspergillus* species isolated from different cheese types were identified and recorded in (Fig. 4). The results revealed that *A. niger* could be isolated from the examined roumy, cheddar and white cheese with percentages of 60%, 40% and 40%, respectively, while in case of *A. flavus*; these

values were 40%, 40% and 24%, respectively. *A. parasiticus* was isolated from roumy, cheddar and white cheese with percentages of 24%, 20% and 20%, respectively, while in case of *A. ochracous*; these values were 12%, 8% and 8%, respectively.

Interestingly, all isolates gave lipolytic activities at 25 °C for 10 days on both tween 80 and

tributyrin agar media and with respect to proteolytic activity, all strains showed activity on skimmed milk agar at 25° C for 10 days with different clear zone of casein hydrolysis as shown in (Table. 1).

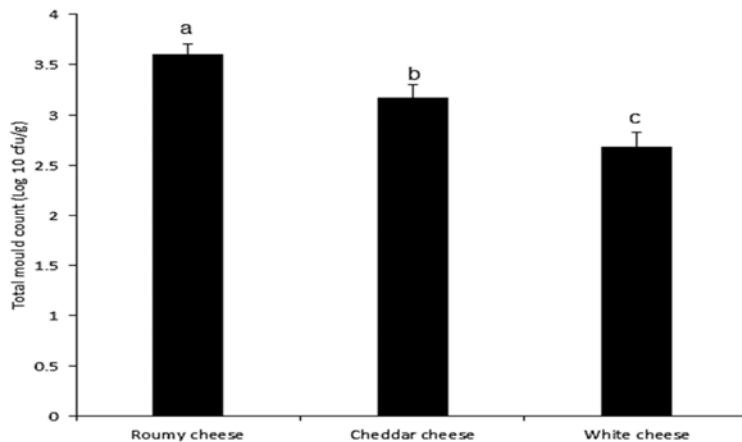


Figure 1. Total mould count in different cheese types marketed in Zagazig city. Total mould count in the different examined cheese types marketed in Zagazig city, Egypt. Values represent means \pm SD (\log_{10} cfu/g) of twenty-five samples for each type and each sample was measured in duplicates. Columns with different letters differ significantly at $P < 0.05$.

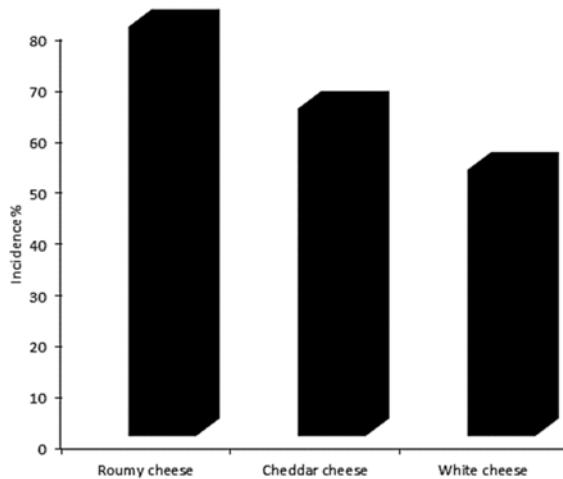


Figure 2. Incidence of mould contamination in different cheese types marketed in Zagazig city. Incidence percentage (%) of the mould contamination in the examined cheese samples ($n = 25$ /each).

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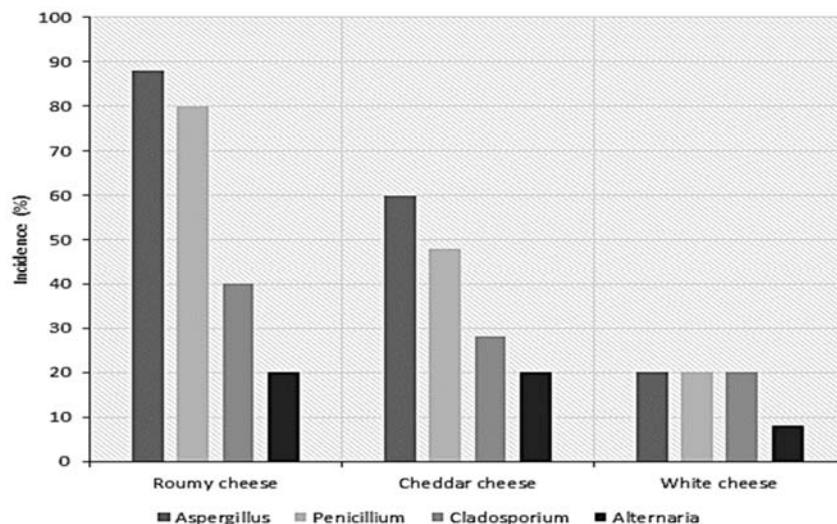


Figure 3. Incidence (%) of different mould genera isolated from different cheese types marketed in Zagazig city

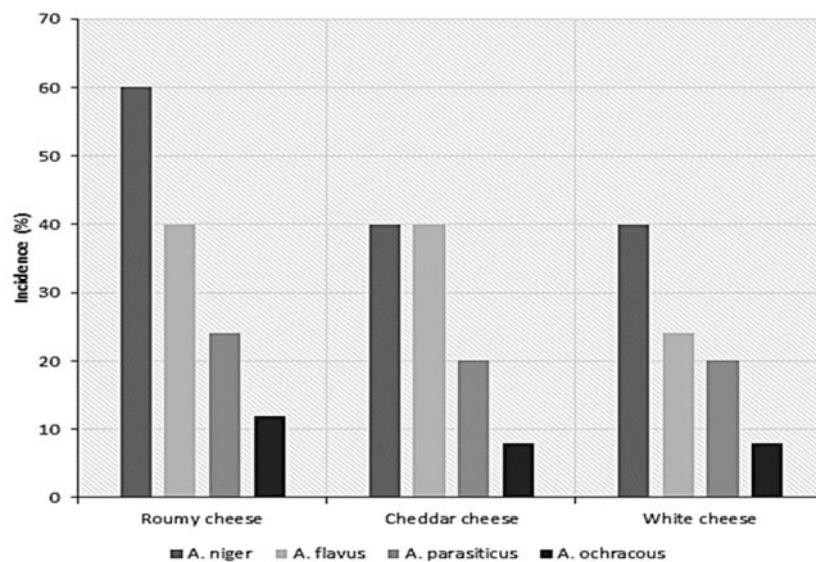


Figure 4. Incidence (%) of different Aspergillus species isolated from different cheese types marketed in Zagazig city.

Table 1: Lipolytic and proteolytic activities of the isolated moulds

Mould species	Lipolytic activity		Proteolytic activity
	Tween 80	Tributyrin	Z.H
<i>A. niger</i>	+ve	+ve	8
<i>A. flavus</i>	+ve	+ve	14
<i>A. parasiticus</i>	+ve	+ve	12
<i>A. ochraceous</i>	+ve	+ve	2
<i>Penicillium sp.</i>	+ve	+ve	10
<i>Cladosporium sp.</i>	+ve	+ve	10
<i>Alternaria sp.</i>	+ve	+ve	6

Z.H =Zone of casein hydrolysis in millimeter; A = Aspergillus.

4. DISCUSSION

Dairy products like cheese and others represent an important part of the food menu not only in Egypt but also in the other parts of the world. Thus, investigating the food-borne microbiological hazards due to consumption of such products is a matter of a great importance. Among all microbiological hazards, moulds had received very little attention.

In this study, the incidence of mould contamination of cheese marketed in Zagazig city, Egypt was investigated that rouny cheese had significantly higher TMC followed by cheddar and white cheese and this TMC was in correspondence with the incidence percentage of mould contamination.

In line with these results, about 60% of cheese samples in Slovenia were contaminated with moulds, but the concentration of moulds was much lower in the cheese ($2.1 \log_{10} \text{cfu/g}$) than our report, which is probably due to the consequence of the contamination from the environment (Torkar and Vengus, 2008). Furthermore, they calculated the incidence percentage and they recorded that moulds were found in 10 (71.4%) out of 14 of curd samples, in 9 (69.2%) out of 13 soft cheese samples and in 5 (38.4%) out of 13 semi-hard cheese samples. The contamination with moulds in samples of curd and soft cheese was 2.8 and 2.2 $\log_{10} \text{cfu/g}$, respectively, and was much higher than the contamination level of semi-hard cheese samples (mean value $1.3 \log_{10} \text{cfu/g}$). Higher numbers of yeasts and moulds in cheese samples were also recorded (Godic Torkar and Golc Teger, 2004).

Mould contamination of dairy products in this study indicates inadequate sanitary measures performed during processing of such products. The conditions of the environment in the manufacturing rooms, stores, refrigerators and shops are very suitable for the development of moulds inside the products, but more frequently on the surface of various sorts of cheese (Jay 1992). Serious contamination takes place from soil and water, raw milk becomes contaminated from milk contact surfaces, equipment, utensils, handling by workers and during transportation (Godic Torkar and Vengus, 2008).

Concerning the isolated and identified moulds; *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* could be isolated with different percentages from the examined samples (Fig. 3). Mainly *Aspergillus* and *Penicillium* were the dominant isolated moulds while *Cladosporium* and *Alternaria* were less dominant

Furthermore, we identified the different isolated *Aspergilli* and found that *A. niger*, *A. flavus*, *A. parasiticus* and *A. ochraceous* were the dominant *Aspergillus* species while *A. parasiticus* and *A. ochraceous* were less dominant, as clear in (Fig. 4).

This finding goes in accordance with the findings of Scott (1989) as well as of Finne Kure *et al.* (2004), who reported that genus *Penicillium* was most frequent mould in cheese, followed by *Aspergillus*, *Cladosporium*, *Geotrichum* and *Mucor*. In cheese samples, there are only a few different sorts of moulds because the pasteurization reduces the presence of contaminants from the group of yeasts and moulds. However, *Penicillium* and *Aspergillus* can grow over a wide range of pH from 2 to 11; over a water activity value ranges from 0.620 to 0.995; over a temperature ranges from -10 to around 60 °C and over a wide range of nutrient limitations (Pitt and Hocking 2009), these reasons may explain the frequent isolation of these two moulds from milk products in Egypt and other countries. We further examined all the isolated moulds from the examined cheese samples for their lipolytic and proteolytic activities.

From these results shown in table (1), it notes worthy that, mould growth may give rise to changes in different organoleptic parameters of the end cheese products. In line with these findings, Asefa *et al.* (2009) mentioned that mould contamination is often associated with unpleasant appearance, color, odor and changes in taste and nutritional value of foods. These changes may lead to significant economic losses due to unmarketability of the products.

Regarding to public health hazards of the isolated moulds, generally, mould growth may introduce a meat product to consumers containing aflatoxins, ochratoxin and other mould metabolites like antibiotics and allergens, which represent a potential health hazard to consumers too. Mycotoxins are secondary metabolites that are toxic to vertebrates when introduced via food through natural route. Ochratoxin A, which can be immunosuppressive, nephrotoxic, teratogenic, and has been classified as a possible carcinogen to human (Darwish *et al.*, 2014).

In conclusion, mould contamination of cheese marketed in Zagazig city, Egypt, was clearly observed in this study indicating unsatisfactory hygienic measures adopted during cheese manufacture. The prevalent mould species were *Aspergillus* and *Penicillium*. The growing moulds may represent potential health hazards for consumers. Thus, strict hygienic measures should be followed during the processing and manufacture of different dairy products in Egypt to avoid the unwanted mould growth in the final milk products.

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