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Studies on mold contamination of retailed cheese in Zagazig city, Egypt

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ABSTRACT

Cheese is considered as a healthy and complete nutrient that supports part of the human needs with protein, minerals, and vitamins. This study was conducted to examine the mold growth in four cheese types including Kariesh, Feta, Domiati, and Rumy retailed in Zagazig city, Egypt. In addition, isolation and identification of the different mold genera was further screened. Furthermore, the proteolytic and lipolytic abilities of the identified molds were screened. Rumy cheese samples followed by Kariesh cheese samples showed the highest total mold count. The prevalent mold genera were *Aspergillus spp.*, *Penicillium spp.*, *Cladosporium spp.*, and *Fusarium spp.* Identification of the Aspergillus isolates revealed five *species*, namely, *A. niger*, *A. flavus*, *A. fumigatous. A. ochraceous*, and *A. versicolor*. The detected molds had clear *in-vitro* lipolytic and proteolytic activities. The public health significance of the isolated molds was discussed. Therefore, strict hygienic measures should be adopted during all manufacture steps of these kinds of cheese.

1. INTRODUCTION

Cheese is a major source for many nutrients including essential amino acids, calcium magnesium, niacin, and vitamins A, and B12 (Gerosa and Skoet, 2013; Ma et al., 2020). The microbiological quality of cheese is a major determinant for the shelf life of cheese (McSweeney, 2007).

Mold contamination of milk and dairy products is of a particular importance in the field of food industry. Mold growth in cheese and other dairy products might lead to deterioration and spoilage of the final product. Many factors affect the mold growth in food such as moisture, pH, oxygen, substrate, and the interaction with other microbiological agents. Generally, molds can grow over a wide range of pH, temperature, and water activity (Pitt and Hocking, 2009).

Mold growth on the surface of cheese is a common problem during aging and storage of different cheese types. Mold growth over the surfaces of dairy products is implicated in the unmarketability of such products, and subsequently severe economic losses. Several mold genera such as *Aspergillus spp.*, and *Fusarium spp*., can produce toxic metabolites and carcinogenic compounds named as mycotoxins (Darwish et al., 2014).

The present study was performed to estimate total mold counts on four cheese types (Kariesh, Feta, Domiati, and Rumy) mostly consumed in Zagazig city, Egypt.

In addition, isolation, and identification of different mold genera of the examined the retailed cheese samples were done. Furthermore, identification of the dominant Aspergillus species was further studied. The proteolytic and lipolytic abilities for the identified mold genera were additionally screened. The public health significance of the identified mold genera was discussed.

2. MATERIAL AND METHODS

2.1. Sample collection

Eighty cheese samples of Kariesh (Raw soft cheese), Feta, Domiati (White cheese 6% salt), and Rumy (Ras cheese), (20 of each) were collected from different stores in Zagazig city, Egypt. The mycological examination of cheese samples was done at Faculty of Veterinary Medicine, Zagazig University.

2.2. Sample preparation

Twenty-five grams from each sample was blended aseptically in buffered peptone water 0.1% (225 ml) for 2 min at 2500 rpm to obtain a dilution of 10^{-1}, followed by making decimal serial dilutions (APHA, 2001).

2.3. Determination of total mold count (TMC)

Total mold counts (TMC) were determined by the pour plate technique using both malt extract agar media for ordinary molds and Czapeck-Dox agar with 5% NaCl for xerophilic molds (Oxoid, Basingstoke, UK) followed by incubation in dark at 25ºC for 5-7 days (APHA, 2001).

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2.4. Identification of the isolated molds

Identification of molds was conducted according to the protocol of Pitt and Hocking (2009) using the macroscopical and microscopical characteristics of the mold colonies.

2.5. Evaluation of lipolytic and proteolytic activities of the existed molds

Effect of lipase activity on Tween 80 was done (Kotula et al., 1982) and on Tribytrin (Alford, 1976). Proteolytic activity was investigated according to Harrigan and McCance (1966).

2.6. Statistical analysis

Statistical analysis was done using Tukey–Kramer HSD test where, $p< 0.05$ indicated statistical differences (Gomez and Gomez, 1984).

3. RESULTS

The obtained results in the present study revealed mold contamination of the examined cheese types at variable percentages. Rumy cheese showed the highest mold contamination at 75% followed by Kariesh cheese and Domiati cheese at 50% and 30% respectively. The lowest mold growth was seen in feta cheese at 20% (Fig. 1).

Total mold counts (log 10 cfu/g) were estimated at the examined cheese types. Rumy cheese had the highest average TMC 3.28 ± 0.19, followed by Kariesh samples with 2.66 ± 0.46, Domiati cheese and Feta cheese with 2.21 ± 0.24 and 2.08 ± 0.15 log 10 cfu/g, respectively (Fig. 2).

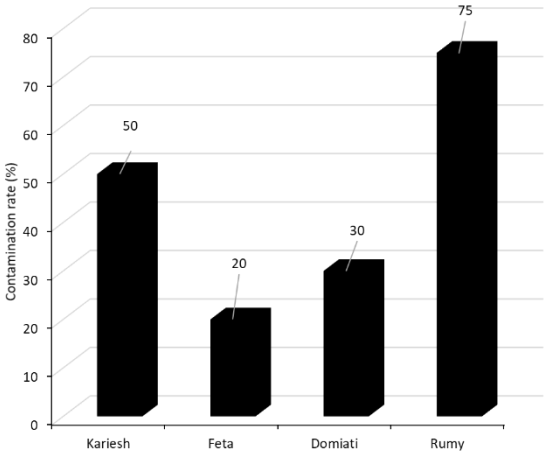

Fig. 1 Mold contamination rates (%) among cheese types (Kariesh, Feta, Domiati, and Rumy) retailed in Zagazig city, Egypt (n = 20).

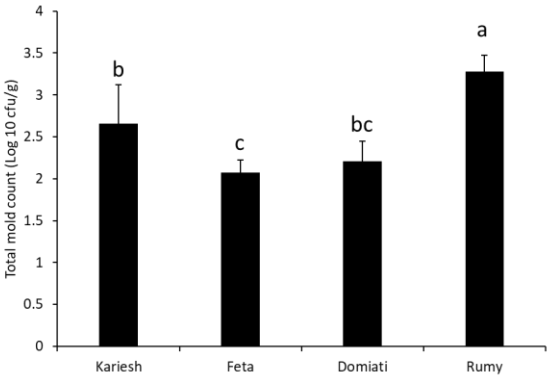

Fig. 2 Total mold counts (log10 cfu/g) of the examined cheese types (Kariesh, Feta, Domiati, and Rumy) retailed in Zagazig city, Egypt. Values represent means ± SD (n = 20). Columns with different letter were statistically different at p< 0.05.

Four mold genera could be identified in the current study including *Aspergillus spp.*, *Penicillium spp.*, *Cladosporium spp.*, and *Fusarium spp*. The predominant mold genera among the identified molds were *Aspergillus spp.*, which showed prevalence rates of 21.92%, 17.81%, 9.59% and 2.74% in Kariesh cheese, Rumy cheese, Domiati cheese, and Feta cheese, respectively.; followed by *Penicillium spp.*, with prevalence rates of 8.22% and 13.70% in Kariesh and Rumy cheese, respectively. At the same time, the prevalence rate of Penicillium was similar in Domiati and Feta cheese at 4.11% (Fig. 3).

Five *Aspergillus spp*. were identified in the present work, namely, *A. niger, A. flavus, A. fumigatous, A. ochracous,* and *A. versicolor*. The predominant *Aspergillus spp.* among the different cheese types were *Aspergillus niger,* (Kariesh cheese (15.78%), Rumy cheese (13.15%), Domiati cheese (5.26%), and Feta cheese (5.26%), followed by *A. flavus,* (Kariesh cheese (15.78%), Rumy cheese (13.15%), and Domiati cheese (7.89%) (Fig. 4).

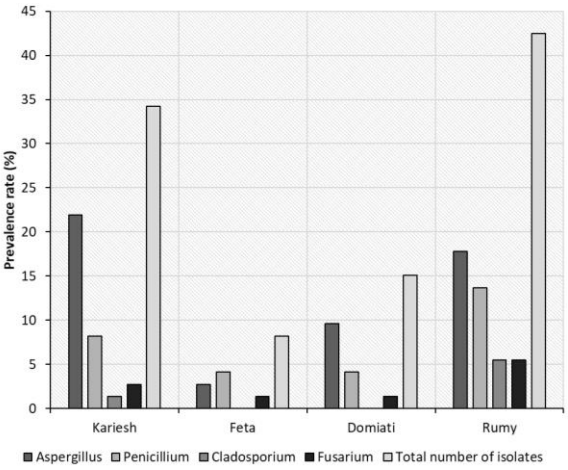

Fig. 3 Prevalence rates (%) of the identified mold genera among the examined cheese types (Kariesh, Feta, Domiati, and Rumy) retailed in Zagazig city, Egypt.

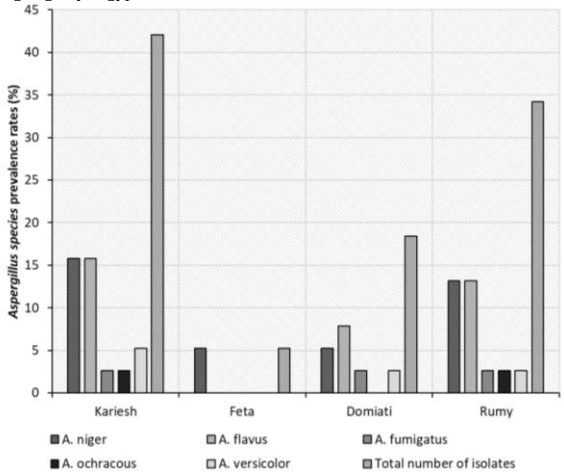

Fig. 4 Prevalence rates (%) of the identified *Aspergillus spp.*, among the examined cheese types (Kariesh, Feta, Domiati, and Rumy) retailed in Zagazig city, Egypt.

All isolated molds from the examined cheese samples had lipolytic activities at 25 °C for 10 days on both tween 80 and tributyrin agar media. With respect to proteolytic activity, all identified isolates showed activity on skimmed milk agar at 25 °C for 10 days with clear zones of casein hydrolysis as shown in Table 1.

Table 1 Lipolytic and proteolytic activities of the isolated molds

Mold species	Lipolytic activity		Proteolytic activity
	Tween 80	Tributyrin	Z.H
A. niger	+ve	+ve	10
A. flavus	+ve	+ve	14
A. fumigatus	+ve	+ve	12
A. ochracous	+ve	+ve	6.0
A. versicolor	+ve	+ve	6.0
Penicillium spp.	+ve	+ve	10
Cladosporium spp.	+ve	+ve	10
Fusarium spp.	+ve	+ve	8.0

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

4. DISCUSSION

Cheese is among the essential dairy products that supply part of the human needs from bioactive peptides, vitamins, and minerals. In the present study, the mold contamination of the retailed cheese in Zagazig city, Egypt was examined. Rumy cheese had the highest contamination rate followed by Kariesh cheese. One possible reason for the high mold contamination in Rumy cheese is the long time of the ripening process (Hassan et al., 2019). In agreement with our results, Ibrahim *et al.* (2015) reported nearly similar mold contamination rates and counts in Kariesh and Domiati cheese retailed in dairy markets in Cairo city, Egypt. However, higher mold contamination and total mold counts was reported for the examined Kariesh cheese retailed in Alexandria city (contamination rate is 94.44%; TMC 7.95±6.00 log 10 cfu/g) (Salem *et al*., 2016). Furthermore, Hassan *et al*. (2019) reported a 100% mold contamination for Kariesh cheese, and 80% for Ras cheese with counts of 5.26±4.96 log 10 cfu/g in Kariesh cheese and 54.67 ± 22.04 cfu/g in Ras cheese retailed in Assuit city, Egypt.

From the possible reasons for the mold contamination of cheese the use of inferior quality raw milk for the manufacture of cheese, the poor hygienic measures adopted during the processing and storage of cheese and the fluctuation of the keeping temperature during distribution (Pitt and Hocking, 2009). In agreement with such assumptions, Weinstein (1991) reported that poor personal hygiene caused more than 90% of the sanitary problems in the food service industry. Poor hygiene at dairy products manufacturing facilities resulted in higher contamination, which may be due to dirty walls, cutting boards, unhygienic handling, and lack of knowledge of hygienic practices (Tambekar et al., 2008).

Aspergillus spp., and *Penicillium spp.*, were the most predominant mold genera in the present study. This could be due to their ability to grow over a wide range of temperatures, their needs for a low oxygen concentration for growth (Plahar et al., 1991). *Cladosporium spp., and Fusarium spp.*, were also identified as they could survive at severe adverse conditions such as low temperatures of up to -7°C and minimal water activity (0.85) (Jay, 2000). *A. niger* and *A. flavus* were the dominant *Aspergilli*. It is noteworthy that *A. flavus* is one of the major aflatoxigenic molds.

From the adverse effects of the mold growth on the dairy products is their massive economic losses due to production of off flavor and colors on the final product and subsequently reducing their shelf life and marketability. Although all examined cheese samples in the present study had normal sensory characteristics, the lipolytic and proteolytic activities of the identified molds were further examined *in-vitro*. The obtained results revealed that all identified molds had lipolytic and proteolytic activities as indicated in Table (1). Similarly, Habashy et al. (2019) recorded significant lipolytic and proteolytic activities for *Aspergillus spp.*, and *Penicillium spp in-vitro.*

There are many adverse health effects for the isolated molds. For instances, *A. niger* is implicated in case of pulmonary Aspergillosis and produce toxic metabolites such as kojic acid, oxalic acid and malformins (Bennett, 1980). *A. flavus* is implicated in the craniocerebral Aspergillosis, allergic bronchopulmonary Aspergillosis, and produce toxic metabolites like aflatoxins, aspergillic acid, kojic acid, asperotoxin, cyclopiazonic acid, and sterigmatocystin (Chakrabarti et al., 2002; Hedayati et al., 2007). *A. fumigatous* is implicated in aspergillosis, aspergilloma, allergic reactions, and produce toxic metabolite called gliotoxin (Hohl and Feldmesser, 2007). *A. ochraceous* produces ochratoxin A, and citrinin (Darwish et al., 2014). *A. versicolor* produces sterigmatocystin (Kamei and Watanabe, 2005). *Penicillium spp.* produces many toxic metabolites such as meleagrin (mutagenic), roquefortine C (neurotoxic), mycophenolic acid (Immunosuppressive), penitrem A (tremorgenic), and terrestric acid (cardiotoxic) (Pitt and Hocking, 2009). *Fusarium spp.* produces ochratoxins and deoxynivalenol mycotoxins (Darwish et al., 2014). Cladosporium spp. produce several allergens (Schoch et al., 2009)

5. CONCLUSION

In conclusion, the obtained results demonstrated the presence of mold contamination in the retailed examined cheese in Zagazig city, Egypt which might lead to several public health hazards. Therefore, strict hygienic measures should be adopted during processing, storage, and handling of different cheese types with selection of raw materials of high quality.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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