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*(Original Article)*

# Mycoflora of Some Cheese Types in Assiut City, Egypt

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## Abstract

Ten samples of different types of cheese were collected from local markets in Assiut city. The samples included (four soft cheese, three Ras cheese and three Roquefort cheese). All samples were collected and kept under refrigeration condition (5 – 7 ºC) Czapek's dextrose agar medium used for isolation and identification the mycoflora of them. The results observed that nine species belonging to the genera *Aspergillus, Penicillium, Alternaria, Eurotium* and *Rhizopus* were recovered and identified. *Aspergillus* was the most predominant and represented by four species namely *A. flavus*, *A. niger*, *A. paraciticus*, and *A. fumigatus*. *Euotium* represented by two species namely *E. repens* and *E. chevalieri*. *Aspergillus niger* Found in the four types of cheese. This resreach amied to determine the chemical and microbiological quality of some types of cheese including detection and identification of fungi.

***Keywords****: soft cheese, Roquefort cheese, Ras cheese, chemical and microbiological quality, mycoflora.*

## Introduction

Cheese is considered as an essential daily food for many people around the world. It has a nutritional value that provides an essential source of digestible protein and minerals, including calcium and phosphorus (Hammam *et al*., 2020). Cheese ripening is a complex and dynamic biochemical process that includes protein breakdown, fat hydrolysis and lactose metabolism. These processes are catalyzed by agents such as residual coagulant, indigenous milk enzymes, starter or nonstarter microflora and secondary organisms. The secondary organisms include moulds and presence of moulds on the surface of mould-ripened cheese gives them a different appearance and flavor from other cheeses. The moulds have more complex enzymatic systems than bacteria and their enzymes contribute to cheese maturing of the cheese, i.e., to proteolysis and lipolysis which are more extensive in these cheeses (Hayaloglu and Kirbag, 2007).

Cheese, although they have been characterized as one of the safest food products by some authors (Little *et al*., 2008), in 2006 the consumption of contaminated cheese accounted for the 0.4% of the total foodborne outbreaks in

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Europe (EFSA, 2008). Furthermore, the scientific literature has reported several food poisoning outbreaks associated with various types of cheese. While traditional soft white cheese is considered healthy, it could be a good medium for infectious microbes. The risk of contamination is a problem internationally and not restricted to one area. The Food and Drug Administration (FDA) referred that some raw milk cheeses are a likely cause for health problems. Consumption of cheese contaminated with undesirable microbes was the source for 0.4% of the overall foodborne epidemics in Europe (Abdulghani and Kareem, 2019).

The fungal diversity which presented in cheese depends on the microbial quality of milk, heat treatment of milk, manufacturing temperature, humidity during ripening, amount of salting, and microbial contamination during and after manufacture (Torkar and Teger, 2006). Microbial contamination of cheese may originate from various sources. Such sources during cheese production might be starter culture, brine, floor and packaging material, cheese cloth and curd cutting knife, cold room and production room air (Temelli *et al.*, 2006). *Penicillium, Aspergillus, Cladosporium, Alternaria, Fusarium* and *Talaromyces* are most frequently reported genera in cheese spoilage (Pamela *et al*., 2019; Panelli *et al*., 2012 and Masotti *et al*., 2019). In addition, some adventitious molds can also contaminate cheese during the ripening process (Petersen *et al*., 2010). Contaminating cheese with fungi lead to spoilage or produce undesirable flavors, aromas, or other metabolic products which rendering them unsuitable for consumption (Banjara *et al*., 2015). The growth of toxigenic fungi during ripening of cheese must be considered as a problem of safety for human consumption. During the ripening of cheese, non-toxigenic strains of fungi should be avoided, moreover, fungi growth on the cheese surface causes economic losses and quality problems. Fungi are significant spoilage microorganisms of foodstuffs during the storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Fungal growth on cheese is a common problem for the cheese manufacture during ripening and curing as well as for the retailer and consumer during refrigeration storage. Species of *Penicillium* and *Aspergillus* are common contaminants of cheese (Gandomi *et al.,*2009).

This work aims to isolation and identification of fungi growing on the Egyptian cheese in Assiut city.

## Material and Methods

A total of 10 samples of cheese were collected from different shops in Assiut city. All samples put in ice tank 5 ºC and transfer immediately to the laboratory for further analysis.

## Chemical analysis

Cheese samples were analyzed for titratable acidity, total nitrogen and soluble nitrogen, fat content and ash were determined as described in AOAC (2012). Salt content in cheese samples was determined as described by Pearson (1975).

## Microbiological analysis

### Preparation of Samples

Ten grams of cheese were weighted and emulsified in sterile mortar 90 ml sodium citrate solution 2%. This 1:10 of cheese used for making serial dilutions required for the microbial analysis.

## Microbiological analysis

### The total viable counts (TVCs)

An aliquot (1 ml) of diluted material was plated on agar medium and incubated for 48 hours at 37ºC. Each dilution was plated in duplicated plates and plates containing 30-300 colonies were considered (Marshall, 2004).

### Yeasts and Moulds

One ml of the appropriate dilution was plated on potato dextrose agar medium and incubated at 28-30ºC for 6 days in moulds (Smith and Dawson, 1944) and one ml in the appropriate dilution was plated on yeast- malt extract ager medium and incubated at 24-26ºC for 3 days in yeast (Wickerham, 1951).

### Coliforms incidences

Coliforms incidences were determined by the inoculation of dairy samples or their dilution into Mac Conkey broth (Mohran, 1971).

### Statistical analysis

Data of all treatments were statistically analyzed using Statistix 8.1 software (Analytical software, 2003) and subjected to the analysis of variance under complete randomized design. Means were separated by Least Significant Difference test (LSD test) at a significant level of 0.05.

## Results and Dissection

**Table 1. Gross chemical composition of different types of cheese**

| Types of cheese | Cheese properties Mean+SD | | | | | |
|---|---|---|---|---|---|---|
| | Titratable Acidity | Fat content | Total nitrogen | Soluble nitrogen | Salt content | Ash |
| Ras cheese | 0.84 ±0.11[a] | 31.67 ± 2.16[a] | 3.42 ±0.32[a] | 0.41 ±0.05[b] | 5.097 ±0.493[b] | 6.63 ±0.65[b] |
| Requfort cheese | 0.740 ±0.078[b] | 32.33 ±2.066[a] | 3.50 ±0.14[a] | 1.25 ±0.05[a] | 4.340 ±0.729[b] | 4.08 ± 0.52c |
| Kareish cheese | 0.440 ±0.125[c] | 2.000 ±0.894[c] | 2.42 ± 0.22[b] | 0.35 ±0.04[c] | 2.912 ±0.370[c] | 2.84 ± 0.27[d] |
| Baramili (Domiati) | 0.48 ±0.104[c] | 21.67 ±1.862[b] | 2.11 ± 0.09[c] | 0.02 ±0.01[d] | 5.445 ±0.845[a] | 7.22 ± 0.25[a] |

Means with the same letter are not significantly different ($p \leq 0.05$)

Data in Table 1 represented the mean values of titratable acidity, fat content, total nitrogen, soluble nitrogen and salt content of different types of cheese which collected from local market in Assiut city. It could be observed that the highest value of titratable acidity was for Ras cheese with 0.84±0.11% as lactic acid

followed by Roquefort cheese with 0.740±0.078, while the lowest values was for Baramiely cheese with 0.48±0.104. However, there are no significant differences in acidity content between Kareish cheese and Baramiely cheese ($P \leq 0.05$), but while was significant between Ras cheese and Roquefort cheese. From the same Table it was observed that the fat content of Roquefort cheese recorded the highest mean values with (32.33±2.066%) followed by Ras cheese with (31.67±2.16%) , while the Kareish cheese recorded the lowest mean value with (2.00±0.894%). The results showed significant differences between all cheese verities ($P \leq 0.05$). From the data presented in the previously mentioned table could be observed also that both of total nitrogen and soluble nitrogen of Roquefort cheese were of higher values than others cheese with 3.50±0.14 and 1.25 ±0.05, followed by Ras cheese with 3.42 ±0.32 and 0.41 ±0.05%, respectively.

The examined samples of Baramiely cheese showed the lowest mean values of total nitrogen and soluble nitrogen with 2.11 ±0.09 and 0.02 ±0.01%, respectively. Statistically it was observed that the differences between total nitrogen and soluble nitrogen in all studied cheese were significant ($P \leq 0.05$). As with the salt content tabulated in the previous Table showed that the Barameily cheese had the highest value of salt of 5.445 ±0.845%, followed by Ras cheese of 5.097 ±0.493%, while the lowest value of salt percentage was for Kareish cheese 2.912±0.370%. From the statistical analysis it was observed there is a significant difference between different type of investigated cheeses ($P \leq 0.05$). From previous finding it was concluded that the differences in the chemical composition of different type of investigated cheeses may be related to the difference in starter culture used in its manufacture, the repining period, storage condition, moisture percentage and the salt contents in its manufacturing procedure which reflects on its final chemical composition.

**Table 2. Microbiological analysis of different types of cheese**

| Types of cheese | Cheese microbiological properties ($log_{10}$ cfu/gm) Mean +SD | | | | |
|---|---|---|---|---|---|
| | Total bacterial counts | Mould counts | Yeasts | Total Yeast &Mould | Coliform bacteria group incidence |
| **Ras cheese** | 6.57 | 2.31 | 2.12 | 4.53 | 66.66% |
| **Roquefort cheese** | 6.39 | 2.56 | 3.01 | 5.14 | ND |
| **Kareish cheese** | 7.08 | 2.48 | 0 | 4.48 | ND |
| **Baramiely cheese** | 7.10 | 2.44 | 2 | 4.58 | ND |

ND: means not founded in the same column.

Data in Table 2 represents the mean values of some microbiological properties of different types of cheese collected from Assiut city. The results indicated that the total bacterial counts log of cheese 6.57 $log_{10}$ cfu/gm, 6.39 $log_{10}$ cfu/gm, 7.08 $log_{10}$ cfu/gm and 7.10 $log_{10}$ cfu/gm for Ras cheese , Roquefort cheese, Kareish cheese and Baramiely cheese, respectively. It was observed from these data that the total bacterial counts were closely related to each other, to the extent that there are no statistically significant differences between the investigated cheese types. Total yeasts and moulds were calculated and the results indicated

that, Roquefort cheese recorded the highest total yeasts and moulds log with mean values of 5.14 $log_{10}$ cfu/gm followed by Baramiely cheese with mean values of 4.58 $log_{10}$ cfu/gm. The difference between the investigated cheese types in their contents of total yeasts and moulds may be related to the different parameters and conditions of during its manufacture procedure and during storage period. as well as the cheese were tested for the incidence of coliform bacteria, the obtained results indicated that, coliform bacteria group had not detected in all cheeses varieties , except for Ras cheese which about 66.66% of the investigated samples confirm the presence of coliform bacteria.

**Table 3. Total counts (CFU per gm Ras cheese), Percentage of total counts (calculated per total counts), number of cases of isolation (out of 3 samples) and occurrence remarks of cheese contaminated fungi isolated from 3 samples of cheese collected from three locations in Assiut on Czapek's dextrose agar medium (CzDA) at 28±1ºC**

| Samples | Ras cheese1 | | Ras cheese2 | | Ras cheese3 | | TOTAL | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Fungal Species*** | **T.C** | **%T.C** | **T.C** | **%T.C** | **T.C** | **%T.C** | **T.C** | **%T.C** | **OR** | **NCI** |
| ***Alternaria chlamydospora*** | 400 | 28.6 | 0 | 0.0 | 0 | 0.0 | 400 | 14.3 | L | 1 |
| ***Aspergillus Niger*** | 150 | 10.7 | 650 | 92.9 | 500 | 71.5 | 1300 | 46.4 | H | 3 |
| ***Aspergillus Fumigatus*** | 800 | 57.1 | 0 | 0.0 | 150 | 21.4 | 950 | 33.9 | M | 2 |
| ***Eurotium repens*** | 50 | 3.6 | 0 | 0.0 | 0 | 0.0 | 50 | 1.8 | L | 1 |
| ***Rhizopus stolonifera*** | 0 | 0.0 | 50 | 7.1 | 50 | 7.1 | 100 | 3.6 | M | 2 |
| **Total counts** | 1400 | 100 | 700 | 100 | 700 | 100 | 2800 | 100 | | |
| **No. of genera** | 3 | | 2 | | 2 | | 4 | | | |
| **No. of species & varieties** | 4 | | 2 | | 3 | | 5 | | | |

TC = Total count, %TC = Percentage of total count, NCI = Number of cases of isolation, OR = Occurrence remark (H= High occurrence; more than 2samples out of 3samples, M= Moderate occurrence; 2 samples, L= Low occurrence less than 2 samples).

## Mycoflora isolated from Ras cheese samples

Data in Table (3) revealed that 5 species belonging to 4genera of fungi were isolated from 3 samples of Ras cheese collected from three locations in Assiut city on Czapek's dextrose agar medium (CzDA) at 28±1ºC. The total counts of fungi isolated were 2800 CFU per ml Ras cheese. The results showed that Ras cheese sample number 1 was the richest sample in fungal population giving rise to 1400 (50% of the total counts), followed by Ras cheese sample number 2& 3 giving 700 (25% of the total counts).

*Aspergillus* was the most prevalent genus isolated in all samples of Ras cheese accounting for 2250 of total fungi with TC% 80.36. The highest population of *Aspergillus* was recovered from Ras cheese sample number 1 giving 1400 CFU per ml Ras cheese (62.22% and 50% of total *Aspergillus* and total fungi, respectively), but both Ras cheese samples number 2and 3 had the number of fungi giving 700 (31.11% and 25% of total *Aspergillus* and total fungi, respectively).

From the genus two species were identified, *A. niger* and *A. fumigatus* with moderate occurrence for each (2 out of 3 samples) giving 57.78 % (1300 CFU per gm Ras cheese), and 42.22 % (950 CFU per gm Ras cheese) of the total counts of *Aspergillus.*

*Alternaria chlamydospor* isolated in low occurrence (1 out of 3 samples) from Ras cheese samples number 1giving 400 CFU per gm Ras cheese with 14.28% of the total fungal counts. *Eurotium repenwas* isolated in low occurrence (1 out of 3 samples) from Ras cheese samples number 1 giving 50 CFU per gm Ras cheese with 1.79% of the total fungal counts. *Rhizopus stolonifer* moderate occurrence (2 out of 3 samples) from Ras cheese samples number 2 & 3 giving 50 and 50 CFU per gm Ras cheese with 1.78 and 1.78% of the total fungal counts.

**Mycoflora isolated from Roquefort cheese samples**

Data in Table (4) revealed that 4 species belonging to 3 genera of fungi isolated from 3 samples of Roquefort cheese collected from three locations in Assiut city on Czapek's dextrose agar medium (CzDA) at 28±1°C. The total counts of fungi isolated were 1550 cfu/ gm Roquefort cheese. The results showed that Roquefort cheese sample number 1 was the richest sample in fungal population giving rise to 650 (41.93 % of the total counts), followed by Roquefort cheese samples number 3 giving 550 (35.48% of the total counts), while the lowest sample in fungal population Roquefort cheese sample number 2 giving 350 (22.58% of the total counts).

*Penicillum* was the most prevalent genus isolated in high occurrence according to the number of cases of isolation (all samples) accounting for 1100 of total fungi with TC% 66.67. The highest population of *Penicillum* was recovered from Roquefort cheese sample number 1 giving 550 cfu/ gm Roquefort cheese (50 and 35.48% of total *Aspergillus* and total fungi, respectively), whereas the lowest population was emerged in Roquefort cheese sample number 2 giving 250 cfu/gm Roquefort cheese (22.73 and 16.13% of total *Aspergillus* and total fungi, respectively).

*Aspergillus niger* was isolated in moderate occurrence (2 out of 3samples) from Roquefort cheese samples number 1 and 2 giving 100 cfu/gm Roquefort cheese with 6.45% of the total fungal counts. *Aspergillus parasiticus* was isolated in moderate occurrence (1 out of 3samples) from Roquefort cheese samples number 3 giving 150 cfu/gm Roquefort cheese with 9.68% of the total fungal counts. *Rhizopus stolonifer* was isolated in low occurrence (1 out of 3 samples) from Roquefort cheese sample number 3 giving 100 cfu/ gm Roquefort cheese with 6.45% of the total fungal counts.

**Table 4. Total counts (CFU per gm Roquefort cheese), Percentage of total counts (calculated per total counts), number of cases of isolation (out of 3 samples) and occurrence remarks of cheese contaminated fungi isolated from 3 samples of cheese collected from three locations in Assiut on**

| Samples | Roquefort cheese 1 | | Roquefort cheese 2 | | Roquefort cheese 3 | | TOTAL | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Fungal Species** | **T.C** | **%T.C** | **T.C** | **%T.C** | **T.C** | **%T.C** | **T.C** | **%T.C** | **OR** | **NCI** |
| ***Aspergillus niger*** | 100 | 15.4 | 100 | 28.6 | 0 | 0.0 | 200 | 12.9 | M | 2 |
| ***Aspergillus parasiticus*** | 0 | 0.0 | 0 | 0.0 | 150 | 23.1 | 150 | 9.7 | L | 1 |
| ***Penicillium roqueforti*** | 550 | 84.6 | 250 | 71.4 | 300 | 46.15 | 1100 | 70.9 | H | 3 |
| ***Rhizopus stolonifera*** | 0 | 0.0 | 0 | 0.0 | 100 | 18.2 | 100 | 6.5 | L | 1 |
| **Total counts** | 650 | 100 | 350 | 100 | 550 | 100 | 1550 | 100 | | |
| **No. of genera** | 2 | | 2 | | 3 | | 3 | | | |
| **No. of species & varieties** | 2 | | 2 | | 3 | | 4 | | | |

TC = Total count, %TC = Percentage of total count, NCI = Number of cases of isolation, OR = Occurrence remark (H= High occurrence; more than 2samples out of 3samples, M= Moderate occurrence; 2 samples, L= Low occurrence less than 2 samples).

Data in Table 5. revealed that 3 species belonging to 2 genera of fungi were isolated from 2 samples of Karish cheese collected from two locations in Assiut city on Czapek's dextrose agar medium (CzDA) at 28±1°C. The total counts of fungi isolated were 1000 cfu/ gm Karish cheese. The results showed that Karish cheese number 1 was the richest sample in fungal population giving rise to 550 (55% 0f the total counts), while the total count of Karish cheese sample number 2 giving 450 (45% 0f the total counts). *Aspergillus* was the most prevalent genus isolated from the two Karish cheese sample accounting 700 of the total fungi with TC% 70. The highest population of *Aspergillus* was recovered from Karish cheese sample number 2 giving 500 cfu/ gm Karish cheese (71.43% and 50% of total *Aspergillus* and total fungi, respectively), whereas the lowest population was emerged in Karish cheese sample number 2 giving 200 (28.57% and 20 of total *Aspergillus* and total fungi, respectively). From the genus two species were identified, *A. niger*, and *A. flavus* with moderate occurrence for each (two samples) giving 64.29% (450 cfu/ gm Karish cheese), and 35.71% (250 cfu/ gm Karish cheese) of the total counts of *Aspergillus. Eurotium chevalieri* was isolated in moderate occurrence (All samples) giving 50 and 250 cfu/ gm Karish cheese with 5% and 25% of the total fungal counts.

**Table 5. Total counts (cfu/ gm Karish cheese), Percentage of total counts (calculated per total counts), number of cases of isolation (out of 2 samples) and occurrence remarks of cheese contaminated fungi isolated from 2 samples of cheese collected from 2 locations in Assiut on Czapek's dextrose agar medium (CzDA) at 28±1°C.**

| Samples | Karish cheese 1 | | Karish cheese 2 | | TOTAL | | | |
|---|---|---|---|---|---|---|---|---|
| **Fungal Species** | T.C | %T.C | T.C | %T.C | T.C | %T.C | OR | NCI |
| ***Aspergillus niger*** | 450 | 81.8 | 0 | 0.0 | 450 | 45 | L | 1 |
| ***Aspergillus flavus*** | 50 | 9.1 | 200 | 44.4 | 250 | 25 | M | 2 |
| ***Eurotium chevalieri*** | 50 | 9.1 | 250 | 55.6 | 300 | 30 | M | 2 |
| **Total counts** | 550 | 100 | 450 | 100 | 1000 | 100 | | |
| **No. of genera** | 2 | | 2 | | | 2 | | |
| **No. of species & varieties** | 3 | | 2 | | | 3 | | |

TC = Total count, %TC = Percentage of total count, NCI = Number of cases of isolation, OR = Occurrence remark (H= High occurrence; more than 2samples out of 3samples, M= Moderate occurrence; 2 samples, L= Low occurrence less than 2 samples).

## Mycoflora isolated from baramili cheese samples

Data in Table (6) revealed that 2 species belonging to 1 genera of fungi were isolated from 2 samples of Baramili cheese collected from two locations in Assiut city on Czapek's dextrose agar medium (CzDA) at 28±1°C. The total counts of fungi isolated were 450 cfu/ gm Baramili cheese. The results showed that Baramili cheese sample number 2 was the richest sample in fungal population giving rise to 350 (77.78% of the total counts), followed by Baramili cheese sample number 1 giving 100 (22.22% of the total counts).

**Table 6. Total counts (cfu/ gm Baramili cheese), Percentage of total counts (calculated per total counts), number of cases of isolation (out of 2 samples) and occurrence remarks of cheese contaminated fungi isolated from 2 samples of cheese collected from two locations in Assiut on Czapek's dextrose agar medium (CzDA) at 28±1°C.**

| Samples | Baramili cheese 1 | | Baramili cheese 2 | | TOTAL | | | |
|---|---|---|---|---|---|---|---|---|
| **Fungal Species** | T.C | %T.C | T.C | %T.C | T.C | %T.C | OR | NCI |
| ***Aspergillus flavus*** | 0 | 0.0 | 350 | 100 | 350 | 77.8 | L | 1 |
| ***Aspergillus niger*** | 100 | 100 | 0 | 0.0 | 100 | 22.2 | L | 1 |
| **Total counts** | 100 | 100 | 350 | 100 | 450 | | | |
| **No. of genera** | 1 | | 1 | | 1 | | | |
| **No. of species & varieties** | 1 | | 1 | | 2 | | | |

TC = Total count, %TC = Percentage of total count, NCI = Number of cases of isolation, OR = Occurrence remark (H= High occurrence; more than 2 samples out of 3 samples, M= Moderate occurrence; 2 samples, L= Low occurrence less than 2 samples).

*Aspergillus* was the most prevalent genus isolated in high occurrence according to the number of cases of isolation (All samples) accounting for 450 0f total fungi with TC% 100. The highest *Aspergillus* was recovered from Baramili cheese sample number 2 giving 350 cfu/ gm Baramili cheese (77.78% of total *Aspergillus* and total fungi, respectively), whereas the lowest population was emerged in Baramili cheese sample number 1giving 100 cfu/ gm Baramili cheese (22.22% of total *Aspergillus* and total fungi, respectively). From the genus two species were identified, A. flavus, and A. niger, with moderate occurrence for each (All

samples) giving 77.78% (350 cfu/ gm Baramili cheese, and 22.22% (100 cfu/ gm Baramili cheese) of the total counts of *Aspergillus.*

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# الميكروفلورا من بعض أنواع الجبن في مدينة أسيوط بمصر

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## الملخص

تم جمع عشـــر عينات من أنواع مختلفة من الجبن من الأســـواق المحلية بمدينة أســـيوط. وشـملت العينات (اربعة عينات جبن طري، ثلاثة عينات راس جبن، ثلاثة عينات جبن ريكفورد). تم جمع جميع العينات وحفظها تحت ظروف التبريد (5-7 درجة مئوية). اسـتخدمت بيئة تشـابكس دكستروز اجار لعزل وتعريف الفطريات منها. أوضحت النتائج أنه تم اكتشاف وتحديد تسعة أنواع تنتمي إلى أجنـاس *Aspergillus* و *Penicillium* و *Alternaria* و *Eurotium* و *Rhizopus*. هي Aspergillus الأكثر انتشارًا وتمثلت بأربعة أنواع هي *A. flavus* و *A. niger* و *A. paraciticus* و *A. fumigatus*. يمثل *Euotium* نوعين هما *E. repens* و *E. chevalieri*. *Aspergillus niger* توجد في الأربعة الأنواع من الجبن.