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Molecular diagnosis of some species of local cheese contamination with fungi in Tikrit City

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mold, yeast, food spoilage, canned, aspergillus

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ABSTRACT
In the current study, 60 samples of different cheeses were examined to determine the extent of fungal contamination. The results showed the presence of mold in 24 samples (40%) and yeast in 16 samples (26.6%). The results of the local white cheese showed that this type of cheese had the highest percentage of mold contamination, reaching 33.3%, and the presence of yeast in it was 2.3%. *Aspergillus fumigatus* recorded the highest percentage of contamination, representing 62.5% of the total mold contamination in the cheese in the current study. *Aspergillus fumigatus* and *Aspergillus niger* were the most contaminating mold species in the studied cheese, representing 25% of the total mold. *Aspergillus flavus* and *Mucor circinelloides* accounted for 12.5%. The study showed the presence of *Pencillium roqueforti* and *Rhizopus stolonifer* in approximately 8.33% of the examined samples. *Acremonium falciforme* and *Pencilium commune* were less common, with an average prevalence of 4.16% across all cheeses. The most prevalent yeast in cheese was *Debaryomyces hansenii*, accounting for 43.75% of the cheeses studied, followed by *Rhodotorula mucilaginosa*, at approximately 25%, and *Candida lipolytica*, at 18.75%. *Candida albicans* was less common, accounting for 12.5% of all samples. This study confirmed the identity of the two fungal species using BLAST analysis, achieving 100% accuracy for Aspergillus fumigatus and 99% accuracy for *Mucor circinelloides*, with one gene mutation observed. These results confirm the importance of genome sequencing in fungal classification and evolutionary relationships.

التشخيص الجزيئي لبعض أنواع الجبن المحلي الملوث بالفطريات في مدينة تكريت

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الخلاصة

ف ي الدراسة الحالية تم فحص 60 عينة من أنواع مختلفة من الجبن لتحديد مدى التلوث الفطري. أظهرت النتائج وجود العفن في 24 عينة (40٪) والخميرة في 16 عينة (26.6٪). أظهرت نتائج الجبن الأبيض المحلي أن هذا النوع من الجبن كان لديه أعلى نسبة تلوث بالعفن، حيث بلغت 33.3٪، ووجود الخميرة فيه كان 2.3٪. سجل *Aspergillus fumigatus* أعلى نسبة تلوث، حيث حوالي 62.5٪ من إجمالي تلوث العفن في الجبن في الدراسة الحالية. كان *Aspergillus fumigatus* و *Aspergillus niger* أكثر أنواع العفن تلويثًا في الجبن المدروس، حيث مثلا 25٪ من إجمالي العفن. شكل *Aspergillus flavus* و *Mucor circinelloides* نسبة 12.5٪ . أظهرت الدراسة وجود *Pencillium roqueforti* و *Rhizopus stolonifer* في حوالي 8.33٪ من العينات المفحوصة.وتبين أن كلاً من فطري Acremonium falciforme و*Pencilium commune* كانا أقل شيوعًا، بمتوسط انتشار بلغ 4.16% في جميع أنواع الجبن. وكانت الخميرة الأكثر انتشارًا في الجبن هي *Debaryomyces hansenii*، حيث شكلت 43.75% من أنواع الجبن المدروسة، تليها *Rhodotorula mucilaginosa*، بنسبة تقارب 25%، ثم *Candida lipolytica* بنسبة 18.75%. وكانت *Candida albicans* أقل شيوعًا، حيث شكلت 12.5% من جميع العينات. وأكدت هذه الدراسة هوية النوعين الفطريين باستخدام تحليل BLAST، محققةً دقة 100% لفطر *Aspergillus fumigatus* و99% لفطر *Mucor circinelloides*، مع ملاحظة طفرة جينية واحدة. وتؤكد هذه النتائج أهمية تسلسل الجينوم في تصنيف الفطريات والعلاقات التطورية.

الكلمات المفتاحية : اعفان ، خمائر ، تلف اغذية ، اجبان معلبة ، جبن محلي ابيض ، الرشاشيات

INTRODUCTION

Food loss through spoilage and waste during production is one of the most significant global concerns facing humanity in relation to food security and safety (Fao F. A. O. S. T. A. T, 2018). Since primitive man began to store food and cultivate crops, toxic fungi began to spoil food, and signs of food spoilage appeared, including spots of different colours black, red, and green, the appearance of fuzz, acidity, and disintegration, resulting in the production of harmful and spoiled products (Adebayo, *et al.*,, 2014). Concerning food spoilage, food products can be biochemically, chemically, physically, or microbiologically spoiled. Fungi and/or Bacteria are the main agents that cause microbial spoilage (Pitt & Hocking ,2009) .Until now, it was generally believed that fungi only cause unwanted spoilage of food, despite the fact that some of them have been associated with human diseases for two hundred years, and acute poisoning by fungi, which are microorganisms, has been known for a long time. The world has become aware that simple molds can cause serious toxins and problems. Molds cause digestive disorders and liver diseases in humans due to their ability to form (mycotoxins), so they have a serious impact on the general health of humans and animals. Fungi affect the physical properties of food (taste and smell) and chemical properties (pH and texture of food) and this leads to a decrease in the quality of food (Alcano *et al.*,,2016). Studies indicate that fungi account for between 5% and 10% of total food loss and spoilage in developing countries(Pattono *et al.*,, 2013) The quantity of cheese discarded owing to fungal deterioration is substantial (Martin *et al.*,, 2021). Fungi are the primary agents of food

degradation due to their ability to proliferate on diverse substrates and in conditions unsuitable for other microorganisms. Fungal deterioration of cheese constitutes a significant issue, resulting in diminished quality due to both apparent and latent flaws. Certain fungi that develop on cheese may generate mycotoxins, presenting a food safety concern. Notwithstanding attempts to mitigate contamination, fungal deterioration of cheese adversely affects the economy through product degradation, diminished quality, increased labor, and food safety concerns, particularly when mycotoxins are generated (Taniwaki, 2018).

The fungal genera responsible for cheese poisoning are varied and many such several as Rhizopus, Acremonium, Aureobasidium, Aspergillus, Alternaria, , Wallemia, Botrytis, Epicoccum , Cladosporium, Exophiala , Lecanicillium, Eurotium, , Fusarium, Gliocladium , Penicillum and Mucor). Nevertheless, the most responsible genus for food and cheese spoilage is Penicillium followed by Aspergillus (Garnier *et al.,,* 2017; Marín *et al.,,* 2015). Mycotoxins are a heterogeneous group of toxic secondary metabolites produced by toxigenic fungi that contaminate a wide range of cheeses, cereals, fresh vegetables, cocoa, nuts, fruits before harvest and during storage, etc. There are many derivatives of these toxins, which may reach more than 600 species. The mycotoxins that can cause frequent contamination of human foods and animal and plant products are aflatoxins (B1, B2, M1, G1, G2), fumonisins (B1, B2, G2), ochratoxin A, trichothecenes (dexenvalenol, T2 toxins, HT2 toxins, nevvanilol), zearalenone, penicillic acid, and patulin are considered important (Alshannaq *et al.,,* 2016; Kępińska-Pacelik & Biel, 2021 ; Zain , 2011)

Cheese contamination by fungi occurs at different stages of production. Milk is usually heat-treated before cheese making or pasteurization, as most fungi are generally heat-sensitive, and milk is therefore not a source of contamination (Garnier, *et al.,,* 2017). However, heat-resistant fungi and their spores can cause spoilage of heat-treated cheese (Pitt and Hocking, 2009). Some local cheeses are produced from unpasteurized milk, where fungal growths on raw milk later appear as fungal threads and discoloured spots on the cheese (Marín *et al.,,* 2015). An important source of cheese contamination is air. Airborne spores can contaminate cheeses in preparation tanks and vats. Fungal and mold spores on the surface of cheeses can grow visibly if the conditions are favorable. Cheese that is brought and produced without packaging is sensitive to contamination from air (Barrios *et al.,,* 1998).

Yeasts are organisms that play a major role in cheese spoilage and depending on the species (Atanassova *et al.,,* 2016; Buehler *et al.,,* 2017). yeasts are widely distributed and are often found in raw milk, production surfaces, brine, cheese tub, air, curd cutting knife and cloth (Sharaf *et al.,,* 2014; Banjara *et al.,,* 2015). The growth of yeasts in cheese in large numbers leads to reduced shelf life, altered sensory properties and deterioration of the quality of dairy products (Radha and Nath, 2014). In addition to various negative effects on cheese quality, such as discoloration, undesirable flavours, bulging of the case, and softening of the cheese texture (Geronikou *et al.,,* 2020; Cardoso *et al.,,* 2015; Garnier *et al.,,* 2017). The proliferation of yeasts in cheeses depends on several factors, such as availability of nutrients, production and storage conditions, milk composition and interactions with commensal microorganisms (Soliman and Aly, 2011; Buehler *et al.,,* 2017).

MATERIAL AND METHODS

Sample collection

Sixty cheese samples were collected between September 1, 2024, and October 1, 2024. The samples were divided into fifty samples of local cheese from different markets in Tikrit (local Arabic cheese), and ten samples of different types of cheese available in Iraqi markets, with three replicates for each type. These samples were collected using sterile forceps, placed in sealed polyethylene plastic bags, placed in refrigerated containers for sample transport, and transported directly to the laboratory for culture and diagnosis. (Hayaloglu & Kirbag , 2007)

Sample preparation

One gram of cheese sample was added to 9 ml of 2% saline solution in test tubes to be homogenized by vortex device and ready for cultivation in Petri dishes on Potato Dextrose Agar (PDA) which is a selective agar medium used for counting and isolating molds and yeast in dairy products and adding the antibiotic chloramphenicol to prevent bacterial growth and then incubating the dishes at 25 °C for 7 days (Oyet *et al.*,,2020)

Preparation of glass slides

Glass slides were prepared from the grown fungi for the purpose of diagnosis. A part of the colony was taken at the age of 7 days using a sterile loop, and transferred to the glass slide and mixed with a drop of 70% ethyl alcohol. After the alcohol dried, a drop of Lactophenol Cotton Blue (LPCB) Stain wasو of 40X.(Pitt and Hocking, 2009)

Fungal (Molds and Yeast) Identification

Fungal species were identified by determining the morphological and microscopic characteristics in terms of colony shape, colour, and texture, type of mycelium, conidia heads, conidia shape, colour and dimensions. A touch was taken from each colony and inoculated using the culture media inoculation technique on the culture medium for diagnosis (Potato dextrose agar (PDA). These colonies were incubated at 25°C and were identified according to the taxonomic keys followed by (Pitt and Hocking 2009; Frisvad and Samson 2004). Yeast identification was traditionally performed using microscopy, colony characteristics, and morphological features (Geronikou *et al.*,, 2020) , and the diagnosis was confirmed using the VITEK-2 device.

Extraction of fungal molecules

We used a Qiagen commercial fungal DNA extraction kit. Using liquid nitrogen, the fungal isolates were ground into a fine powder. Cell membranes were broken down using Proteinase K and Lysis Buffer. DNA was purified by centrifugation, and a Nanodrop instrument set at 260/280 nm was used to measure its concentration and purity.

ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and 18S rRNA-specific primers and the (5'-TCCTCCGCTTATTGATATGC-3') ITS4 were used in thermal PCR

Taq Polymerase, dNTPs, and $MgCl_2$ were all used in the PCR process.Gel Electrophoresis was used to assess the PCR results after samples were processed on a 1.5% Agarose gel and

stained with SYBR Green. The NCBI database was compared with the sequences using the BLAST (Basic Local Alignment Search Tool) methods (Lobo, 2008).

RESULTS AND DISSCUSION

Fungi play a major role in food spoilage because they are able to adapt to many conditions that prevent the growth of other microbes. Cheese spoilage by fungi is a major challenge, resulting in reduced quality due to visual defects, including off-flavors and odors. Some fungi on cheese can also produce mycotoxins, which raise food safety concerns. The current results showed the isolation rates of mold and yeast fungi in different types of cheese (Figures 1,2,3) .

The present search showed that 60 samples of different types of cheese were analyzed to determine the extent of their contamination with fungi and yeast. The results showed the presence of mold in 24 samples at a rate of 40%, and yeast in 16 samples at a rate of 26.6% (Table 1). The results of local white cheese showed that this type of cheese had the highest percentage of mold contamination, reaching 33.3%, and yeast was found in it at a rate of 2.3%. Cheese samples may be contaminated due to unhygienic practices in cheese manufacturing or during transportation and handling, especially in Tikrit, which suffers from energy shortages. However, contamination may occur in animals or cheese samples from the external surfaces of animals or cheese equipment or individuals if proper hygiene practices are not maintained. This study is consistent with another study where local white cheese showed positive results and high levels of mold and yeast were present in it. (Kareem *et al.,,* 2016) Another study confirms and agrees with the results of the current study, which supports the presence of yeast in Arabic white cheese in different proportions. (Khalil *et al.,,* 2018).
Basil cheese contained a molds level estimated at about (3.3%) and yeast (1.6%). While Sulaymaniyah cream cheese and Hema square cream cheese, the two types of samples showed a molds level of about 1.6% and no yeast was recorded. As for the other canned types, as shown in the table below, no fungal contamination was shown compared to the other types in this research. This current study is consistent with another study showing contamination by both molds and yeasts in processed and fresh cheeses. (Atheeb & Maktoof, 2023). Cheese must be manufactured and processed in an atmosphere that meets basic hygiene standards and hygiene management throughout the process in order to maintain its microbiological quality, safety, and shelf life. High contamination rates in fresh cheese may be due to the absence of effective heat treatment or inadequate storage conditions, and should highlight the need for improved production and storage processes. (Fenster *et al.,,* 2019) .

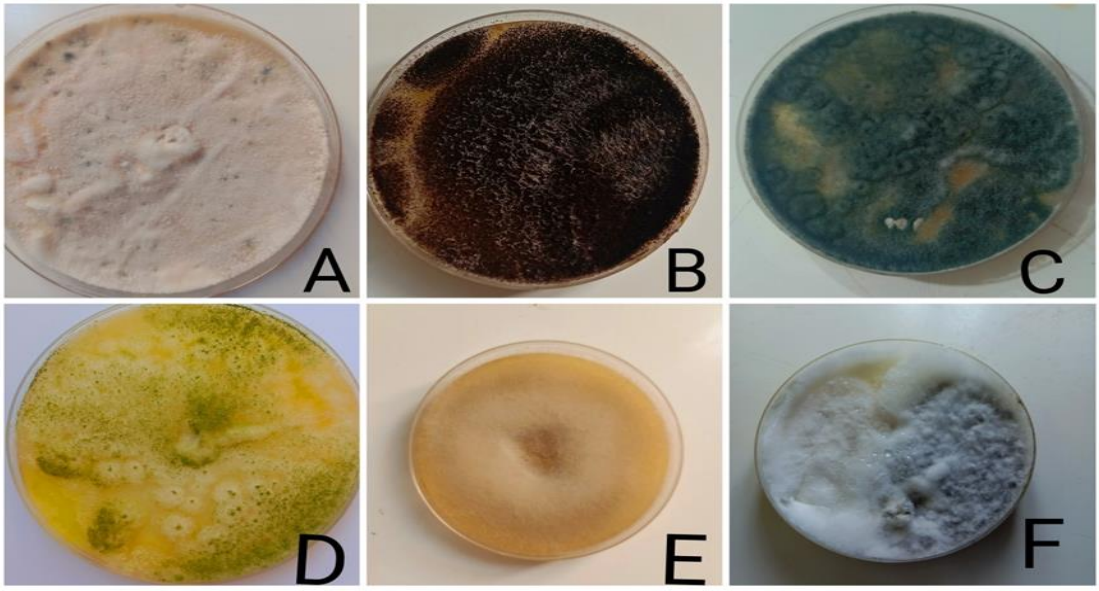

Figure 1. Morphological characterization of some isolated molds

A: ***Acremonium falciforme*** **B:** ***Aspergillus nige*** **C:** ***Aspergillus fumigatus***
D: ***Aspergillus flavus*** **E:** ***Rhizopus stolonifer*** **F:** ***mucor circinelloides***

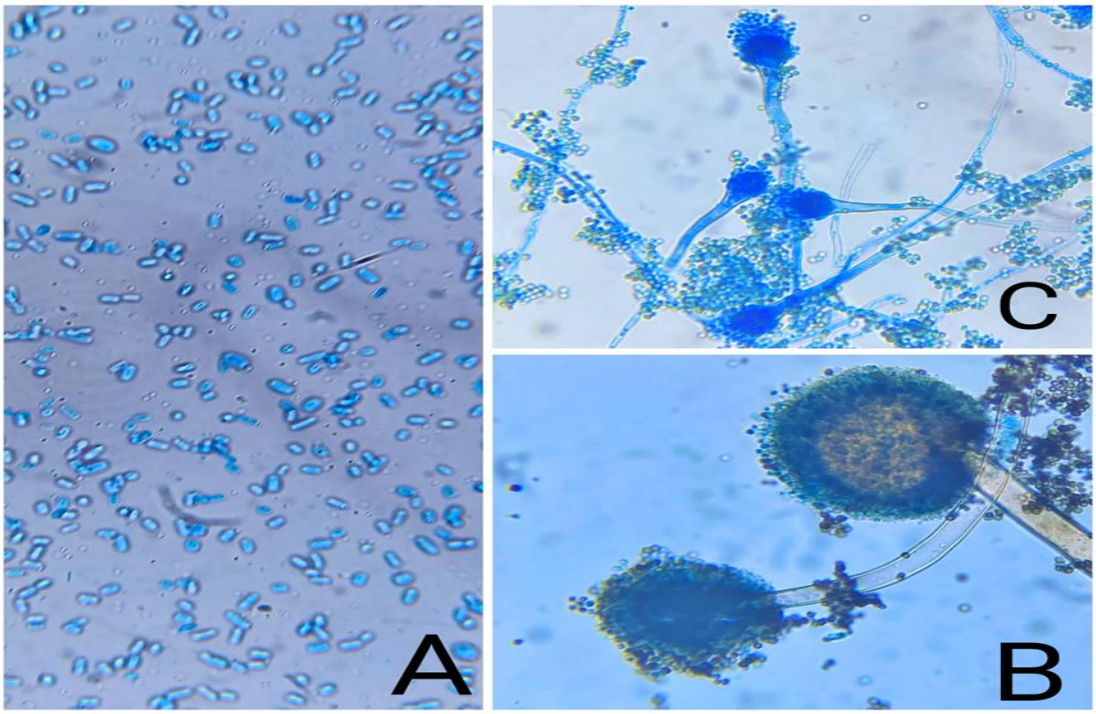

Figure 2 : Microscopic examination of some fungi stained with lactophenol cotton blue stain

A: ***Rhodotorula spp*** **B:** ***Aspergillus niger*** **C:** ***Aspergillus fumigatus***

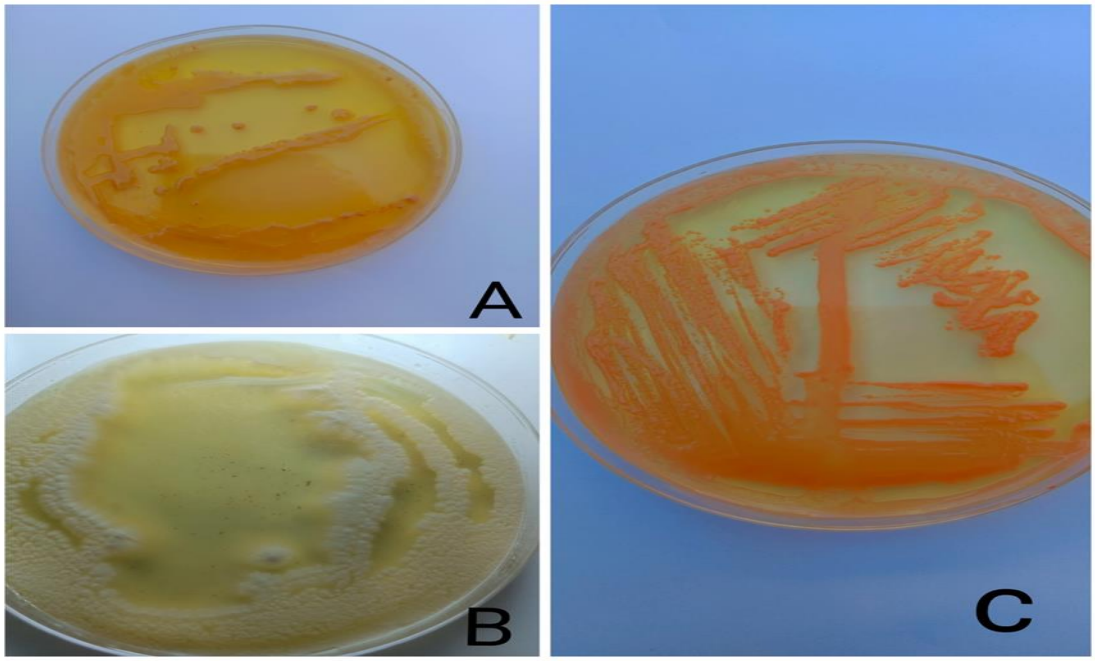

Figure 3: Morphological characterization of some isolated yeasts
A: *Saccharomyces sp* **B: *candida krusei*** **C: *Rhodotorula mucilaginosa***

Table 1: Types of cheese, their Characterizes, and the number of molds and yeasts in them

Samples type	Characterizes	Mold	No.	%	Yeast	No.	%
Local white cheese	Arab cheese from Tikrit markets	+	20	%33.3	+	14	23.3%
Slemani cheese	Cream cheese	+	1	1.6%	-	-	0%
Slemani cheese	Cheddar taste cheese	-	-	0%	-	-	0%
Zahrat alrabea cheese	Pasteurized white cheese	-	-	0%	-	-	0%
Iraqi Mercin cheese	Cheddar cheese	-	-	0%	-	-	0%
Hazar (lamatna) cheese	Natural cheese	-	-	0%	-	-	0%
Chiao cheese	Cheddar cheese	-	-	0%	-	-	0%
Jebnatna cheese	Cheese with basil	+	2	3.3	+	1	1.6%
Hema cheese	Cream cheese squire	+	1	1.6	-	-	0%
Alradidain (abo ghreib)	processed cheddar cheese	-	-		-	-	0%
Jebnatna cheese	Cream cheese	-	-		+	1	1.6%
Total		24		40%	16		26.6%

The most prevalent yeast in cheese in this current study was *Debaryomyces hansenii*. There is a study that is consistent with the current results when this yeast was isolated and was the most prevalent in their study (De Souza *et al.,,* 2021). The percentage of *Debaryomyces hansenii* presence was estimated at 43.75% in all samples (Table 2) , divided into 37.5% in local white Arab cheese, and 6.25% in canned cheese type Jebnatna with basil (Table 3). In the local white Arab cheese, the results showed that the second highest yeast was *Rhodotorula mucilaginosa*, which was isolated at 25%, while *Candia albicans* and *Candida lipolytic* constituted 12.5%. *Candida lipolytic* was present in Jebnatna cream cheese at a rate of 6.25%, and no other yeasts were isolated from the other types of cheeses studied.
A study consistent with the results of the current research found that both *R. mucilaginosa* and *Candida spp.* are the two species that cause cheese spoilage (Geronikou *et al.,,* 2020).
In the samples from which fungi were isolated, the CFU/g of yeasts was higher than 10^5 CFU/g (Table 2), concerning yeast spoilage in dairy products, there is a paucity of evidence on these values. Yeasty and fermented off-flavors were detected when yeast populations attained or above 10^5–10^6 CFU/g (Ledenbach & Marshall, 2009). Sharaf *et al.,* 2014) and (Banjara *et al.,* 2015) indicate that yeasts are frequently present in raw milk, brine, air, production surfaces, cheese vats and cloths, curd cutting tools, and various other objects. The ripening process, aroma compound production, and interaction with starter cultures can be influenced by the incorporation of yeasts as adjunct cultures in specific cheese varieties, including blue-veined and smear-ripened cheeses (Ferreira and Viljoen, 2003; Kesenkaş and Akbulut, 2008; Gori *et al.,,* 2013; Ryssel *et al.,,* 2015). Although yeasts may occasionally be a part of secondary microflora, they are generally regarded as pollutants and are excluded from starter cultures in white cheeses (Kesenkaş and Akbulut, 2008).

Yeast spoilage may adversely affect the sensory attributes, shelf life, and overall quality of dairy products (Salustiano *et al.,,* 2003; Radha and Nath, 2014). The growth of yeast in dairy products is influenced by milk composition, nutritional availability, interactions with commensal microorganisms, and production and storage circumstances (Soliman and Aly, 2011; Buehler *et al.,,* 2017). Moreover, many yeasts are recognized for their distinct spoilage characteristics, attributable to variations in biochemical functions and metabolic activities (Akabanda *et al.,,* 2013; Haastrup *et al.,,* 2018; Bayili *et al.,,* 2019). In this context, ascertaining the genotype of yeast at the strain level and precisely identifying yeast species taxonomically are essential for evaluating the spoilage risk posed by yeast contamination.

The proliferation of yeasts in fresh white cheeses results in undesirable tastes, textural softening, gas generation, discoloration, and package swelling (Geronikou *et al.,,* 2020). The contamination of white fresh cheese with yeasts will diminish its shelf life and compromise its quality. A primary cause of economic losses in the food sector is microbial contamination. Moreover, the proliferation of foodborne microorganisms might result in poisonous food, potentially causing serious illnesses in consumers. Yeasts are recognized as microorganisms that contribute to food rotting. The presence of yeasts in fresh cheeses will result in off-flavours, discoloration, textural softening, and gas production. (Yalcin & Ucar , 2009).

Aspergillus spp fungi recorded the highest contamination rate in the different samples studied, as it constituted 62.5% of the total number of mold causing contamination of cheeses in the current study (Table 2,3). (Silva *et al.,,* 2015) obtained comparable findings, identifying *Aspergillus spp*. as the predominant fungal contaminant responsible for the deterioration of cheese and milk. Aspergillus was identified by (ELbagory *et al.,,* 2014) as the most frequently occurring genus among the tested cheese species samples.

(Table 2) showed the colony forming unit was estimated at 2×10^3 CFU/g and infection rate of *Aspergillus fumigatus* was 25% , which aligns with the findings of another study (Shaker & Farghaly, 2021) and surpasses the findings reported by (Hamed ,2016). *A. fumigatus* in this study appeared about 20.83% and 4.16% in Local white Arab cheese and Jebnatna cheese with basil (Table 3) *.A. fumigatus* is a rapidly growing and decomposing fungus that proliferates extensively and emits thousands of airborne conidia (Croft *et al.,,* 2016). Nonetheless, it can readily disseminate in settings linked to cheese manufacturing during processing, ripening, or storage, resulting in the generation of mycotoxins (Sugui & Kwon-Chung, 2015 ; Ashton & Dyer,2019).

Aspergillus niger was 25% in all cheese samples, divided into 16.66% in local white Arab cheese, and 4.16% Hema Cream squire cheese.The CFU of *A. niger* was 2.5×10^3 CFU/g , this result is completely consistent with the result of another study, where *Aspergillus niger* was isolated at a rate of 25% (Abdullah et al , 2007)**,**
Aspergillus flavus was identified at 12.5% in local white cheese in the present study, which is consistent with another study where this fungus was found at 12.2%. (Seddek *et al.,,* 2016). As for the rest of the cheeses in this study, no contamination with this type of mold appeared on them.

The CFU of *Mucor circinelloides* in study was 2×10^3 CFU/g, the contamination rate of this fungus in local white Arab cheese was estimated at 12.5%, but it did not appear in canned cheeses in this study. *Mucor spp.* is species within the phylum Mucoromycota commonly identified as spoilage fungi linked to milk are significant spoiling agents of concern in fresh or short-ripened cheese matrices (Valle, *et al.,,*2022; Biango-Daniels & Wolfe 2021; Shi & Maktabdar 2022). Certain Mucor species can proliferate in anaerobic environments; however, this does not apply to cheese packaged in vacuum or modified atmospheres. Moreover, they are proficiently adapted to the dairy environment, low temperatures, and pH levels (Shi & Knøchel 2021). *Mucor circinelloides* van tieghem is acknowledged as a spoiling agent in cheesemaking. This fungus is a dimorphic organism capable of transitioning between yeast and fungal stages based on environmental factors (Homa *et al.,,* 2022; Valle, *et al.,,*2023). Numerous Mucor species are recognized as pathogenic. *M. circinelloides* has been identified as a causal agent of mucormycosis in immunocompromised individuals following the consumption of contaminated yogurt (Mueller *et al.,,* 2019; Lee *et al.,,* 2014)

The CFU of *Acremonium falciforme* in the present study was 7×10^2 CFU/g, the presence of this fungus was estimated at 4.16% in local white cheese, its presence has not

been proven in other types of cheese studied. Acremonium species have also been identified, including in cheese produced in Brazil (Martin *et al.,,* 2023).

The percentage of *Pencillium commune* in local white Arab cheese was 4.16%,and the CFU of *P. commune* was 7×10^2 CFU/g. Another study proves that it is a cause of cheese contamination (Hlebová *et al.,,*2022) . The primary environment of *P. commune* is food, especially cheese, in which it is one of the most important contaminants, both in the finished products and in the process of their production (Hayaloglu & Kirbag 2007).

In this study, another type of *Penicillinium fungus* was isolated from the studied samples and it was *Pencillium roqueforti*, this fungus has been isolated at a rate of 4.16% in local white Arab cheese, and 4.16% in Jebnatna Cheese with basil. Several studies and research agree with the current study, *Pencillium roqueforti* was isolated from several types of cheeses (Lund & Frisvad 1995 ; Kure et al .,2001; Kure & Skaar 2000)

The spoilage of local white Arab cheese in the present study *Rhizopus stolonifer* was estimated at about 8.33% and the CFU was 3 × 103 CFU/g .Other studies have shown that this fungus is one of the molds that cause spoilage and contamination of some types of cheese. (Ando *et al.,,* 2012; De Santi *et al.,,* 2010).

It is necessary to refer to the fourth table that we have included to understand each fungus included in the study and to know its locations and causes.

Table 2: Total percentage of Fungal Species and Corresponding CFU/g Values

Fungi type	CFU/g	Total percentage in all cheese types
Acremonium falciforme	7×10^2 CFU/g	1 (4.16%)
Aspergillus flavus	3×10^3 CFU/g	3 (12.5%)
Aspergillus fumigatus	2×10^3 CFU/g	6 (25%)
Aspergillus niger	2.5×10^3 CFU/g	6 (25%)
mucor circinelloides	2×10^3 CFU/g	3 (12.5%)
Pencillium commune	1.5×10^3 CFU/g	1 (4.16%)
Pencillium roqueforti	2×10^3 CFU/g	2 (8.33%)
Rhizopus stolonifer	3×10^3 CFU/g	2 (8.33%)
Candida Albicans	Above 10^5 CFU/g	2 (12.5%)
Candida lipolytica	Above 10^5 CFU/g	3 (18.75%)
Debaryomyces hansenii	Above 10^5 CFU/g	7 (43.75%)
Rhodotorula mucilaginosa	Above 10^5 CFU/g	4 (25%)

CFU :colony-forming unit

CFU= Dilution factor×Volume of the cultured sample

Table 3: Percentages of positive molds and yeasts isolated from different types of cheese

Samples type	Molds types	No.	%	Yeasts type	No.	%
Local white cheese	*Acremonium falciforme*	1	4.16%	*Candida Albicans*	2	12.5%
	Aspergillus flavus	3	12.5%	*Candida lipolytica*	2	12.5%
	Aspergillus fumigatus	5	20.83%	*Rhodotorula mucilaginosa*	4	25
	Aspergillus niger	4	16.66%	*Debaryomyces hansenii*	6	37.5%
	mucor circinelloides	3	12.5%	-	-	-
	Pencillium commune	1	4.16%	-	-	-
	Pencillium roqueforti	1	4.16%	-	-	-
	Rhizopus stolonifer	2	8.33%	-	-	-
Slemani cream cheese	*Aspergillus niger*	1	4.16%	Nil	0	0%
Slemani Cheddar taste cheese	Nil	0	0%	Nil	0	0%
Zahrat alrabea Pasteurized white cheese	Nil	0	0%	Nil	0	0%
Iraqi Mercin Cheddar cheese	Nil	0	0%	Nil	0	0%
Hazar (lamatna) Natural cheese	Nil	0	0%	Nil	0	0%
Chiao Cheddar cheese	Nil	0	0%	Nil	0	0%
Jebnatna Cheese with basil	*Pencillium roqueforti* & *Aspergillus fumigatus*	1 1	4.16% 4.16%	*Debaryomyces hansenii*	1	6.25%
Hema Cream squire cheese	*Aspergillus* niger	1	4.16%	Nil	0	0%
Alradidain processed cheddar cheese (abo ghrib)	Nil	0	0%	Nil	0	0%
Jebnatna cream cheese	Nil	0	0%	*Candida lipolytica*	1	6.25%
TOTAL		24	100%	TOTAL	16	100%

Table 4: Fungi types localization and pathogenicity

Fungi type	Localization*	Pathogenicity**	References
Acremonium falciforme	O	P	Pérez- Cantero& Guarro, 2020
Aspergillus flavus	I,O	OP	Hatmaker, *et al.,*, 2022
Aspergillus fumigatus	I,O	OP	Burks, *et al.,*, 2021
Aspergillus niger	I,O	OP	Poulsen, *et al.,*, 2021
mucor circinelloides	H	P	Yalcin & Ucar 2009
Pencillium commune	I	OP	Guo,*et al.,*,2021
Pencillium roqueforti	I	OP	Guo,*et al.,*,2021
Rhizopus stolonifer	I	P	Baggio, *et al.,*,2016
Candida Albicans	H	OP	Szabó, *et al.,*,2021
Candida lipolytica	I	OP	Yu, *et al.,*, 2024
Debaryomyces hansenii	I	OP	Angulo, *et al.,*, 2020
Rhodotorula mucilaginosa	I	OP	**Cespedes**, *et al.,*,2022

Localization* I=indoor environment, O=outdoor environment , H=Human commensal . Pathogenicity P=pathogen, OP=opportunistic pathogen**

Results of Genetic Sequence Analysis

The gene sequences of two fungal isolates were examined using BLAST to compare the 18S rRNA genes. The findings indicated that the initial sample was classified as *Aspergillus fumigatus*, exhibiting a 100% correspondence with the reference sequence in the GenBank database, but the subsequent sample was recognized as *Mucor circinelloides*, demonstrating a 99% match. The specifics of the BLAST analysis are presented (Table 5). The results indicated a mutation in the second sample, characterized by a nucleotide substitution (Transition) at position 51, where base T was changed to C. This mutation may influence gene function or indicate genetic variation within the species *Mucor circinelloides*. The study's results demonstrate that the BLAST approach is an efficient method for recognizing and classifying fungi using ribosomal RNA sequences. The precise identification of *Aspergillus fumigatus* bolsters the credibility of the employed methodology, given that this fungus is a significant pathogen responsible for aspergillosis. *Mucor circinelloides* was recognized with 99% accuracy, suggesting a minor genetic discrepancy potentially attributable to intraspecific variation or technical error during the identification process. The identified T/C mutation may hold evolutionary significance, potentially influencing the stability or expression of the gene. These alterations may contribute to the identification of subspecies within fungal taxa, necessitating more genetic investigations to comprehend their effects (Figures 4, 5).

This investigation verified the identity of the two fungal isolates by BLAST analysis, achieving 100% accuracy for Aspergillus fumigatus and 99% for Mucor circinelloides, with a genetic mutation observed. These findings underscore the significance of genome sequencing in fungal taxonomy and evolutionary relationships, enhancing comprehension of microbiological diversity

Table 5: Identification of isolated fungi utilizing 18S rRNA gene sequences and comparison with reference databases.

Gene : 18 s ribosomal RNA gene						
N0	Type of substitution	Location	Nucleotide	Source	Sequence of compare	Identities
1	.....	…….	.....	*Aspergillus fumigatus*	ID: KX788286.1	100%
2	Transition	51	T\C	*Mucor circinelloides*	ID: PQ649867.1	99%

```
Sbjct  20   ............................................................  79
Query  61   ccggggaggccttgcgcccccgggcccgcgcccgccgAAGACCCCAACATGAACGCTGTT  120
Sbjct  80   ............................................................  139
Query  121  CTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAACGGATCT  180
Sbjct  140  ............................................................  199
Query  181  CTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA  240
Sbjct  200  ............................................................  259
Query  241  TTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATG  300
Sbjct  260  ............................................................  319
Query  301  CCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCT  360
Sbjct  320  ............................................................  379
Query  361  CTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGG  420
Sbjct  380  ............................................................  439
Query  421  GGCTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCCAGCCGACACCCAACTTTATTTTTC  480
Sbjct  440  ............................................................  499
Query  481  TAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTT  521
Sbjct  500  ........................................  540
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Figure 4: Genetic Sequence Analysis of *Aspergillus fumigatus* samples

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Query  1    ATAATTTTGGCTCGTCCATTATTATCTATTTACTGTGAACTGTATTATTACTTGACGCTT  60
Sbjct  39   ............T...............................................  98
Query  61   GAGGGATGCTCCACTGCTATAAGGATAGGCGGTGGGGATGTTAACCGAGTCATAGTCAAG  120
Sbjct  99   ............................................................  158
Query  121  CTTAGGCTTGGTATCCTATTATTATTTACCAAAAGAATTCAGAATTAATATTGTAACATA  180
Sbjct  159  ............................................................  218
Query  181  GACCTAAAAAATCTATAAAACAACTTTTAACAACGGATCTCTTGGTTCTCGCATCGATGA  240
Sbjct  219  ............................................................  278
Query  241  AGAACGTAGCAAAGTGCGATAACTAGTGTGAATTGCATATTCAGTGAATCATCGAGTCTT  300
Sbjct  279  ............................................................  338
Query  301  TGAACGCAACTTGCGCTCATTGGTATTCCAATGAGCACGCCTGTTTCAGTATCAAAACAA  360
Sbjct  339  ............................................................  398
Query  361  ACCCTCTATCCAGCATTTTGTTGAATAGGAATACTGAGAGTCTCCTGATCTATTCTGATC  420
Sbjct  399  ............................................................  458
Query  421  TCGAACCTCTTGAAATGTACAAAGGCCTGATCTTGTTTAAATGCCTGAACtttttttAA  480
Sbjct  459  ............................................................  518
Query  481  TATAAAGAGAAGCTCTTGCGGTAAACTGTGCTGGGGCCTCCCAAATAATACTCTTTTAA  540
Sbjct  519  ............................................................  578
Query  541  ATTTGATCTGAAATCAGGCGGGATTACCCGCTGAACTTAAGCAT  584
Sbjct  579  ..........................................  622
```

Figure 5: Genetic Sequence Analysis of *Mucor circinelloides* samples

CONCLUSION

Despite the advancements in industrial machinery, food contamination, especially cheese, is a major problem. Fungi and their growth in cheese are among the obstacles related to quality and food safety. The results of this study indicate the presence of diverse types of molds and yeasts in different types of cheeses studied, whether local, open-faced, or packaged. The results emphasize the need to move away from traditional methods and adopt new and advanced strategies to sterilize and preserve food products from contamination. It also emphasizes the need for confirmed sample diagnosis to address problems related to food spoilage.

CONFLICT OF INTEREST

We, the researchers, declare that we have no conflict of interest regarding this research.

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