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Research Article

IDENTIFICATION HEAT-RESISTANT MOLDS FROM MEAT AND DAIRY PRODUCTS ON MARKET SHELVES IN NORTHERN CYPRUS

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ABSTRACT
Food contamination with heat-resistant molds can be a hidden threat that may go unnoticed. Many factors such as product type, hygiene of food industry, storage conditions, water activity, moisture content, pH and high salt concentration of food products are effective on growth of these molds. Hence, deteriorations occur in the structure, taste and odor of food products and cause health hazards and economic damage to the products. In this study, a total of 93 products, 55 milk and dairy products and 38 meat products were analyzed for the presence of molds which are resistant to 80° C heat treatment for 30 minutes. After macroscopic and microscopic examinations, *Penicillium* spp., *Aspergillus* spp. *Cladiosporium* spp., *Geotrichium* spp. and *Bysochlamyces* spp. were identified from the isolated molds. The most frequently observed heat-resistant mold in meat and dairy products was *Penicillium* spp. isolates belonging to *Aspergillus fumigatus* were identified in both meat products and milk and dairy products. Since mold isolates and metabolites observed in food products are of great importance for public health and to avoid economic losses, it would be beneficial to meticulously control microorganisms in food processing industries.

1.Introduction

Contamination of foods by many pathogenic microorganisms such as bacteria, yeast, fungi, viruses and protozoa is known as the cause of foodborne microbiological diseases (Sakuda, 2017). Foodborne microbiological diseases are considered as a major problem affecting public health all over the world (Luksiene, 2021). Among the factors of this global problem is the risk of cross-contamination that food products may be exposed to during the processes from farm to table. For this reason, in order to preserve the quality and safety of food products in food processing industries, microorganism control must be carried out meticulously (Alcaide-Molina et al., 2009; Costa et al., 2017). In addition to microbial inactivation methods such as optimizing water activity and pH, chemical preservatives and high temperature applications have become widespread (Aaliya et al., 2021). Thermal processes are the most widely used processing technology to eliminate both infectious and spoilage microorganisms in food products (especially milk) and to ensure a long shelf life (Dash *et al.,* 2022).

Dairy and/or meat products can be contaminated from many resources such as working surfaces in food processing area, tools and equipment used, the air of the environment, inadequate hygiene of the food handlers, raw materials and ingredients (Abd El-Tawab *et al.,* 2020; Bernardi *et al.,* 2021). It has been reported that approximately 5% to 10% of food products are spoiled by fungi (Pitt and Hocking, 2009). They can be easily dispersed, especially from soil, water and air by the activities of insects. Molds, known as filamentous fungi, have the ability to recycle dead plant and animal remains, but can be harmful to humans by spoiling their food (Rawat, 2015). Many mold species are microorganisms that can grow in the pH range of 3 to 8, can grow in low water activity, require oxygen for their metabolic processes and are generally the cause of airborne spores (Pitt and Hocking, 2009). On the other hand, mold spoilage observed in foods may also pose important problems in food safety such as the production of mycotoxins, which are secondary metabolites that are dangerous to human health, the presence of antibiotics responsible for allergic reactions, and antibiotic resistance (Aydın *et al.,* 2005; Kaynarca *et al.,* 2019; Zadravec *et al.,* 2023). Although most of the foodborne mold and yeast can be inactivated by pasteurization at 70 ° C for 10 minutes, it is mentioned in the literature that a few ascospores or chlamydospore-producing mold species tolerate food processing steps such as drying, heating, and the application of some chemical disinfectants (Kikoku *et al.,* 2008). 4.3-4.9 pH range and high moisture content in dairy products and the tolerance of low pH and high salt concentration in meat products cause the development of filamentous mold (Bernardi *et al.,* 2021). While many molds grow in the pH range of 3-8, a few species can grow at low water activity levels (0.7-0.8) (Rawat, 2015). For this, the number of molds that cause microbial spoilage can be reduced by using two or more different barriers such as regulating the water activity of foods, applying high temperature, adding preservatives, reducing the available oxygen by vacuum or modified atmosphere packaging (Rico-Munoz *et al.,* 2019). In food mycology, correct identification of molds is important in order to prevent the formation of molds that cause spoilage. Thus, appropriate methods and environments are used according to species-specific characteristics such as mycotoxin production potential and physiology of molds (Shi and Maktabdar, 2022). Heat shock should be used to isolate especially heat-resistant molds from the associated microbiota (Rico-Munoz *et al.,* 2015; Houbraken and Samson, 2017). The most commonly observed heat-resistant micromycetes that cause spoilage in foods are; *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., *Byssochlamys fulva*, *Neosartorya fischeri* and *Talaromyces flavus* species (Aydın *et al.,* 2005; Pieckova *et al.,* 2020).

Heat-resistant molds (HRMs) are characterized by the formation of ascospores or similar structures resistant to thermal processes (Rico-Munoz *et al.,* 2019). Ascospores produced by HRMs can develop under ideal conditions such as cultivation and heating media (pH, saccharide, lipid, organic acid, etc. contents) and can cause food spoilage during storage at appropriate temperatures. Thus, HRMs are thought to be effective in the formation of metabolites and mycotoxins that pose a public health hazard (Rico-Munoz, 2017; Kaynarca *et al.,* 2019; Rico-Munoz *et al.,* 2019; Pieckova *et al.,* 2020; Ulusoy *et al.,* 2022). Some heat-resistant fungal species have the ability to produce mycotoxins such as bissoclamaic acid, patulin, bissotoxin A, asymmetric, fischerin, verruculogen, fumitremorgins A and C, eupenifeldin (Ishara and Gunasena, 2020). On the other hand, ochratoxin A (OTA), which is toxic to vertebrates when consumed, is one of the most critical mycotoxins and its presence is mentioned in some animal-derived foods, especially dry-cured meat products (Andrade *et al.,* 2014; Sanchez-Montero *et al.,* 2019). Reducing or eliminating contamination due to ascospores activated by applied heat shock is important in terms of economic losses and public health (Aydın *et al.,* 2005; Rico-Munoz *et al.,* 2019; Pieckova *et al.,* 2020).

Many factors such as the decline in water activity in meat and dairy products, the presence of preservatives, lack of hygiene and sanitation in tools and equipment in food businesses, personnel hygiene, and ambient air play an important role in the formation of heat-resistant molds. Molds that can withstand high temperatures are an important situation that negatively affects both the producer and the consumer in economic and medical terms. Thus, in this study, it was aimed to i) determine the heat-resistant mold species in meat products that processed at/above 80°C, ii) determine the heat-resistant mold species in milk and dairy products that at/above 80°C, iii) estimate the potential food safety problems arising from the presence of heat-resistant molds.

2. Materials and methods

2.1. Materials

In this study, meat products and dairy products offered for sale in supermarkets and butchers in the Northern Cyprus market were analyzed. A total of 93 products, 55 dairy products and 38 meat products, were analyzed for heat-resistant mold contamination. Some of the samples were purchased in their original packaging, while others were delivered to the laboratory in sterile bags or containers. The samples were purchased one day before the experiment date and stored at +4°C in the Food Hygiene and Technology Laboratory of the Faculty of Veterinary Medicine of the Near East University until the analyses were completed.

2.2. Isolation of Heat-Resistant Molds

In literature searches for the detection of heat-resistant molds, it is observed that many methods are applied. All methods are based on the principle of removing all microorganisms other than the molds that resistant to heat treatment. In this study, the direct incubation method was used (Aydın *et al.,* 2005). After the sample packages were opened under aseptic conditions, 5 grams of product were weighed using a scalpel and forceps using a precision balance (BEL, M214AI). The weighed samples were transferred to sterile bags and 45 ml of 0.1% peptone water (Oxoid CM0509) was added to them and homogenized. Then, the samples were transferred to heat-resistant containers and kept in a water bath (Lab Companion, BW-10H) set at 80°C for 30 minutes. After the waiting process, 0.1 ml (10^2) of the sample was inoculated onto Sabouraud Dextrose Agar (Merck, 105438) using a Drigalski spatula by spreading to detect heat-resistant molds. After the inoculation process, the petri dishes were incubated at 25°C for 5-7 days under aerobic conditions without being turned upside down and by moving them as little as possible.

2.3. Identification of Heat-Resistant Molds

After the incubation period, the media in which growth was observed were evaluated. The identification of mold colonies that had completed their development was made by considering their macroscopic and microscopic features. In macroscopic examination; colony appearance (flat, waxy, velvety, cotton or wool clumps), spreading area, whether the face is flat, umbilical, clustered, cracked, ringed, hairy and color were examined from the front and back of the petri dish. In microscopic examination, the structure of the hyphae, whether they have a septum, their shape, whether there are structures responsible for reproduction at their ends and if there are, their structure were examined with the help of a microscope (Demirci and Arıcı, 2006). The diagnosis of molds was made by using Samson et al. (1984) and Hasenekoğlu (1991). Sections were taken from the media in which mold growth was detected with the help of a scalpel and transferred to a slide. A few drops of Lactophenol Cotton Blue (Merck, 113741) were placed on the section and covered with a coverslip. After waiting for a few minutes for the stain to be absorbed, it was examined and identified with a light microscope (X40).

3. Results and discussions

HRM formation in meat and dairy products causes quality losses such as odor and taste in products due to the release of different acids and gases (Rawat, 2015; Abd El-Tawab *et al.,* 2020). High acid applications, addition of various preservatives, and some processing methods (heat application, etc.) are effective in preventing food spoilage. Thus, although many spoilage microorganisms can be removed from the environment, it may not be sufficient to eliminate some microorganism spores (Ishara and Gunasena, 2021). In this study, it was aimed to evaluate the heat-resistant mold growth after samples taken from milk and dairy products and meat products were kept in a hot water bath at 80°C for 30 min. As a result of the analyzes made from samples taken from both milk and dairy products and meat products, the dominance of *Penicillium* and *Aspergillus* species was observed. The results regarding *Penicillium* and *Aspergillus* formation are similar to many studies (Shaltout and Salem, 2000; Brr *et al.,* 2004; Zohri *et al.,* 2014; Abd El-Tawab *et al.,* 2020). When all results were examined, it was detected that *Penicillium* spp. was isolated at a higher rate. HRM counts after heat treatment from petri plates were expressed in terms of CFU per gram.

3.1. Isolation and identıfication of HRM in milk and dairy products

In this study, *Penicillium* spp., *Aspergillus fumigatus*, *Aspergillus* spp., *Byssochlamys* spp. and *Geotrichium* spp. were identified from 55 milk and dairy products. It was observed that a significant proportion of the total number of isolates belonged to *Penicillium* spp. (49%).

Table 1. Isolation and identification results of **HRM in** milk and dairy products

	Samples	N	Positive Samples	Negative Samples	Species				
					***Aspergillus* spp.**	***A. fumigatus***	***Penicilium* spp.**	***Geotrichium* spp.**	***B. fulva***
Butter	**Milk Butter**	5	5 (8.77%)	-			3 (5.26%)	1 (1.75%)	1 (1.75%)
Cheese	**Cottage cheese**	4	4 (7.01%)	-			4 (7.01%)		
	String cheese	1	1 (1.75%)	-			1 (1.75%)		
	Tulum cheese	2	2 (3.50%)	-			2 (3.50%)		
	White cheese	6	4 (7.01%)	2 (3.50%)	1 (1.75%)		3 (5.26%)		
	Ezine cheese	2	2 (3.50%)	-		1 (1.75%)	1 (1.75%)		
	Kashar cheese	4	3 (5.26%)	1 (1.75%)			3 (5.26%)		
	Halloumi cheese	5	2 (3.50%)	3 (5.26%)	1 (1.75%)		1 (1.75%)		
	Labneh cheese	2	2 (3.50%)	-		1 (1.75%)	1 (1.75%)		
	Cheddar cheese	2	2 (3.50%)	-	1 (1.75%)	1 (1.75%)			
Milk	**Soy milk**	1	-	1 (1.75%)					
	UHT milk	6	3 (5.26%)	3 (5.26%)			3 (5.26%)		

	Condensed milk	1	-	1 (1.75%)					
	Milk jam	1	1(1.75%)	-			1 (1.75%)		
Cream	**Cream**	2	2(3.50%)	-			2 (3.50%)		
Yoghurt	**Buttermilk**	1	-	1 (1.75%)					
	Yoghurt	9	4(7.01%)	5 (8.77%)	2 (3.50%)	2 (3.50%)			
	Strawberry	1	-	1 (1.75%)					
Kefyr	**Kefyr**	2	-	2 (3.50%)			1 (1.75%)		
		57	37 (64.91%)	20 (35.09%)	5 (8.77%)	5 (8.77%)	26 (45.61%)	1 (1.75%)	1 (1.75%)

In addition, no growth was observed in 29% of the samples. Milk and milk products in which no heat-resistant mold species were observed were milk, ayran and yogurt. Dairy products with more than one heat-resistant mold species were butter samples. It is thought that microbial growth may be more due to the high moisture content in butter. In the study conducted by Kotzekidou (1997), *Byssochlamys fulva* showed heat resistance in food products exposed to a temperature of 85°C for 20 minutes. In our study, 26 cheese types were studied and of these, *Penicillium* spp. (n=17) and *Aspergillus* spp. (n=6) were isolated. The reason for the contamination of these molds in cheeses was thought to be because of low-quality milk, lack of hygiene practices in the processing plant and/or personnel, and low moisture content. The high acidity present in dairy products such as yogurt and cream prevents the growth of many bacteria while allowing the growth of yeast and mold. Dash *et al.* (2022) mentioned the presence of various heat-resistant *Byssochlamys nivea* ascospores in cheese and dairy products at 92 °C in their study. In addition, mold spores in raw milk can appear as visible molds in cheeses obtained without pasteurization of raw milk (Kure and Skaar, 2019). The results of the milk and dairy products used in the study are presented in Table 1.

3.2. Isolation and identification of HRM in meat products

In this study, in which the presence of heat-resistant molds was investigated in a total of 38 meat products, the presence of *Penicillium* spp., *Aspergillus* spp. and *Cladiosporium* spp. was detected in meat products. No mold species was found in 79% of the total number of isolates. The species frequently observed in the examined samples were *Penicillium* spp. and *Aspergillus* spp. *Aspergillus fumigatus* (5%) and *Cladiosporium* spp. (5%) were detected in ground beef and meatball samples made from ground beef, and *Aspergillus* spp. (3%) was detected in the fish fingers sample. *Penicillium* spp. (8%) was isolated from smoked turkey, Kayseri pastrami and turkey sausage samples. The high salt level, spices and open-air drying used in the production of meat products are the most important causes of mold formation and mycotoxin production (Abd El-Tawab *et al.,* 2020; Zadravec *et al.,* 2023). According to the study conducted by Magistà *et al.* (2017), it was reported that molds belonging to the *Penicillium* and *Aspergillus* species are well adapted to overcome xerophilic and halophilic conditions and especially dominate during the ripening period of dry-cured meat products. The results of the meat products used in the study are presented in Table 2.

Table 2. Isolation and identification results of HRM in meat and meat products

	Samples	N	Positive Samples	Negative Samples	Species			
					***Aspergillus* spp.**	***Aspergillus fumigatus***	***Penicilium* spp.**	***Cladiosporium* spp.**
Meat	**Sujuk**	7	-	7 (18.42%)	-	-	-	-
	Minced meat	5	1 (2.63)	4 (10.52%)	-	1 (2.63)	-	-
	Meatballs	4	3 (7.89%)	1 (2.63)	-	1 (2.63)	-	2 (5.26%)
	Pastrami	1	1 (2.63)		-	-	1 (2.63)	
	Salami	4	-	4 (10.52%)	-	-	-	-
	Sausage	2	1 (2.63)	1 (2.63)	1 (2.63)	-	-	-
	Veal	3	-	3 (7.89%)	-	-	-	-
Chicken	**Ham**	1	-	1 (2.63)	-	-	-	-
Turkey	**Salami**	5	-	5 (13.15%)	-	-	-	-
	Ham	4	1 (2.63)	3 (7.89%)	-	-	1 (2.63)	-
	Sausage	1	1 (2.63)	-	-	-	1 (2.63)	-
Fish	**Croquet**	1	-	1 (2.63)	-	-	-	-
		38	8 (21.05%)	30 (78.95%)	1 (2.63)	2 (5.26%)	3 (7.89%)	2 (5.26%)

Many environmental and biological variables, such as product type, operating environment and storage conditions, can play important roles in the presence of heat-resistant molds (Gougouli ve Koutsoumanis, 2017; Dos Santos *et al.,* 2018; Pieckova *et al.,* 2020).That's why significantly different p and number of colonies were obtained between dairy and meat products. *Aspergillus fumigatus* was identified in both meat and milk and dairy products. *Aspergillus* species are mostly resistant to hot environments and are dominant in environments with reduced water activity (O'Gorman *et al.,* 2009; Samson *et al.,* 2010; Magistà *et al.,* 2017). On the other hand, in addition to being isolated from foods, it has been reported as an indoor air pollutant that can produce various mycotoxins (Dos Santos *et al.,* 2018). The *Penicillium* dominance observed in the results of the study is consistent with other studies especially on meat products (Perrone *et al.,* 2015; Garnier *et al.,* 2017; Pleadin *et al.,* 2017; Sánchez-Montero *et al.,* 2019; Vila *et al.,* 2019; Lešić *et al.,* 2021; Zadravec *et al.,* 2023). This situation is explained by the fact that *Penicillium* type microorganisms are psychro-tolerant and the ripening of these products mostly occurs in the winter months (Lešić *et al.,* 2021; Zadravec *et al.,* 2023). The air and other environmental conditions in the ripening rooms, which are absolute in the product process, can be an important source of *Penicillium* contamination (Alapont *et al.,* 2013). In literature studies, it is reported that although the most heat-resistant species in foods belong to the genera *Byssochlamys, Neosartorya* and *Talaromyces, Penicillium, Hamigera, Monascus* and *Rasamsonia* are followed by ascospore-forming members (Enigl *et al.,* 1993).

4. Conclusions

Textural, visual, odor or taste deteriorations observed in food products are sensory changes that the consumer cannot accept and can occur at any stage of the food production process. Microorganisms such as mold, yeast and bacteria are the main causes of food spoilage. Many factors such as physical and chemical parameters, processing environment and personnel hygiene can help heat-resistant molds survive and reproduce. In this study conducted between 2021-2023, heat-resistant molds isolated from meat and dairy products were *Penicillium* spp., *Aspergillus* spp., *Byssochlamys* spp., *Geotrichium* spp. and *Cladiosporium* spp. The dominant species isolated from both food products was *Penicillium* spp. regular monitoring of mold species and mycotoxins, especially in food products with high consumption, is extremely important for public health and it is thought that such screenings will contribute to the literature. In order to obtain healthy, safe and quality food products, Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) principles must be adopted. In the subsequent study, it is thought that it is necessary to focus on the environmental factors that affect the formation and survival of mold species resistant to high temperatures in foods.

5.References

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