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# Mycological and enzymatic studies on fresh beef meat sold in Taiz City, Yemen

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## ABSTRACT

The mycological analysis of 30 fresh beef meat samples on Czapek's agar at 7º and 28ºC revealed that, heavily contamination with moulds was observed especially at 28ºC. A total of 234 and 400 colonies ⁄ 450 g meat were collected on both temperatures, respectively. Sixty-seven species belonging to 20 genera were identified. Members of *Aspergillus*, *Mucor*, *Penicillium* and *Trichoderma* were the most prevalent fungi. At 7°C was highly spoilage by yeasts fungi, while filamentous fungi predominated at 28°C. The ability of the common fungal isolates to produce protease and lipase enzymes revealed that most of them were positive. Among 152 isolates tested, 103 (67.8%) and 96 (63.2%) could respectively produce these enzymes. Because the deteriorative effects of the above fungi, food should be frequently and routinely analyzed. Also, it is essential to store the meat at lower temperature immediately after slaughtering and during transport and storage to reduce or prevent mould growth.

**Keywords:** Fresh meat; Food spoilage; Protease; Lipase.

## 1. INTRODUCTION

Meats still is, and will remain, part of the staple diet [1]. Meat is considered an important source of proteins, essential amino acids, B complex vitamins and minerals. Due to this rich composition, it offers a highly favorable environment for the growth of microorganisms. The microbiological contamination of meat occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter-houses and retail establishments [2]. Also, a variety of sources including air, water, soil, feces, feed, hides, intestines, lymph nodes, processing equip-ment, utensils and humans, contribute to the microbial contamination of the sterile muscles of healthy animals during slaughter, fabrication, and further processing and handling [3, 4]. Since it is impossible to entirely prevent contamination occurring during slaughter and dressing, some reports evaluated the microbial contamination of exposed meat surfaces at the retail level [5, 6]. The microbiology of meat spoilage has received considerable attention over the years and the characterization of the typical microflora, which develop on different types of meats during storage, has been well documented [7-19].

Enzymes have the property of causing and regulating specific chemical reactions inside or

outside living cells [20]. The major enzymes are protease, lipase, phosphatase, xanthine oxidase and lactoperoxidase [21]. Enzymatic actions are natural process in the muscle cells of the animals after they have been slaughtered and finally end up in meat self deterioration [22].

The present study was planned for the first time in Yemen to assess the fungal load in fresh beef meat, hence the purpose is to study the following: isolation and identification the moulds which contaminate the fresh beef meat in Taiz City, Yemen. The capability of the isolated moulds to produce protease and lipase enzymes was also assessed.

## 2. MATERIALS AND METHODS

### 2.1. Collection of samples

Thirty samples of fresh beef meat were collected randomly from different butchers shops and supermarkets in Taiz City. The samples were placed in sterile plastic bags and transferred in ice-cooled containers (4°C) to the laboratory for immediate fungal analysis.

### 2.2. Isolation and enumeration of fungi

The direct-plating technique [23] was employed. Fifteen pieces of fresh meat (1 gram each) were placed on the surface of three Czapek's agar plates. The plates were kept in a biological oxygen demand (BOD) incubator for 5-7 days at 28±2°C and 8-10 days at 7±2°C. The developing fungal colonies were isolated, identified and maintained on Czapek's agar media. Percentage incidences of fungi were calculated per 15 pieces for each sample.

### 2.3. Medium used for isolation of fungi

Modified Czapek′s Dox agar medium was used in which the 3% sucrose was substituted with 2% glucose. The composition of the medium (g/l) was: glucose 20; $NaNO_3$, 3; $KH2PO_4 \cdot 7H2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $FeSO_4 \cdot 7H2O$, 0.01 and agar, 15. Rose-bengal (1/15000) combined with chloramphenicol (0.5 mg/ml) were used as bacteriostatic agents [24, 25].

### 2.4. Identification of fungal genera and species

Fungi isolated were identified on the bases of macro- and microscopic features following the keys of Raper and Fennell [26], Booth [27], Ellis [28, 29], Pitt [30], Moubasher [31], Domsch et al. [32].

### 2.5. Screening for enzymatic activity of fungal isolates

The common fungal isolates recovered were tested for their abilities to produce extracellular protease and lipase on agar media as follow: three hundreds and seventeen fungal isolates belonging to eighty-three species related to twenty-three genera, commonly isolated in the current work, were tested for their abilities to produce the two enzymes. The fungal proteolytic was tested using a casin hydrolytic medium as employed by Paterson and Bridge [33]. Hydrolysis of the casein results in a clear zone around the fungal colony.

The fungi lipolytic were test using a modified medium of Ullman and Blasins [34] in which Tween 80 (poly oxy-ethylene sorbitan mono oleate) was added instead of Tween 20. The formation of crystals of calcium salt of the oleic acid liberated by the enzyme or as opaque zone surrounding the colony.

## 3. RESULTS AND DISCUSSION

A total of 234 and 400 colonies⁄450 g of filamentous fungi representing 67 species belonging to 20 genera were identified from 30 samples on Czapek's agar at 7 and 28±2ºC (Table 1). Member of *Mucor*, *Penicillium* and *Aspergillus* were the most common fungi. Eight species were new records in Yemen and there are: *Absidia glauca, Cochliobolus geniculata, Mucor fuscus, M. strictus, P.canescens, P. caseicolum, P. raistricki* and *Phoma exigua.* In this respect, Ismail et al. [9] examined fungal contamination of beef carcasses and could isolate 34 fungal genera, represented by 62 species and one variety of which *Aspergillus, Cladosporium* and *Penicillium* were recovered in high incidences.

Also, Farghaly et al. [35] studied the contamination of meat stored in home refrigerators and eleven mould genera could be identified and the most common genera were *Aspergillus, Penicillium*

and *Cladosporium*. Sørensen et al. [36] studied the mycobiota in the processing areas of two different meat products. The diversity of filamentous fungi in the processing areas was high. The main isolated genera were identified as *Aspergillus*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Eurotium*, *Penicillium*, *Phaeoacremonium* and *Phoma*.

Recently, Omorodion and Odu [37] analyzed three different meat samples namely; beef, chicken and pork obtained from Creek road market, Mile 3 market and Rumokoro market for their microbiological quality using differential, selective and routine media. Thirteen fungal isolates covering three genera were isolated and characterized as *Aspergillus* spp., *Mucor* spp. and *Penicillium* spp.

In the current study, *Mucor* was the first common fungus, isolated in high frequency at both incubation temperatures. It was occurred in 63% and 60% of the samples constituting 33.8% and 19.8% of total filamentous fungi, respectively. Of 5 species identified *M. circinelloides* was the most prevalent, emerging in 47% and 53% of sample having 23.5% and 18% of total fungi, respectively. *M. hiemalis* was isolated in low occurrence at 7ºC (17%) and rare at 28ºC (7% of the samples). The remaining *Mucor* species were isolated only at 7ºC in rare frequency of occurrence (Table 1). These results were greatly similar with those obtained by Mizakova et al. [13]. They studied the presence of various moulds in five kinds of fermented raw meat products and noticed that *Mucor* sp. were the most frequently isolated genus. Also, Omorodion and Odu [37] and Asefa et al. [38] reported that *Mucor* spp. were the among most prevalent genera isolated from different meat products.

*Aspergillus* was the second predominant genus isolated in high frequency at 28ºC and moderate occurrence at 7ºC comprising 50.5% and 13.2% of total fungi, respectively. Twenty species were identified of which *A. flavus, A. foetidus, A. fumigatus, A. niger* and *A. terreus* were the most common especially at 28ºC. They occurred in moderate or low occurrence at both temperatures. The remaining *Aspergillus* species were isolated in rare frequency of occurrence at one temperature and while missing at the other (Table 1). Pal and Bagi [39] investigated the occurrence of fungi in various lymph nodes of domestic buffaloes and isolated *A. fumigates*, *A. flavus*, *A. niger* and *A. terreus.* Ismail et al. [9] reported that *Aspergillus* was represented by 13 species and one variety of which *A. flavus* and *A. niger* were of moderate incidences on beef carcasses, while *A. alutaceus, A. fumigatus, A. sydowii, A. terreus* and *A. versicolor* were rare. Robert et al. [40] stated that the most important fungi on meat were: *A. versicolor, A. niger, A. flavus, A. restrictus* and *Eurotium* spp.

*Penicillium* (15 species) occupied the third common fungus isolated in high frequency at 7ºC and in moderate occurrence at 28ºC. The genus was identified from 53% and 37% of the samples contributing 26.1% and 7.3% of total fungi, respectively. However all *Penicillium* species were isolated in rare frequency except *P. chrysogenum* that was isolated in low occurrence at 7ºC. Also, counts of *Penicillium* were higher encountered at low temperature (Table 1). Robert et al. [40] noticed that the most important penicillia on meat were: *P. commune, P. crustosum, P. aurantiogriseum, P. chrysogenum. P. brevicompactum. P. nalgiovense. P. verrucosum. P. glabrum. P. variabile, P. roqueforti.* Laich et al. [12] found that some of the fungi most frequently isolated from fermented and cured meat products such as *Penicillium chrysogenum*. Some genera were isolated in low occurrence on one temperature and rare or absent on the other such as *Alternaria* (6 samples and 2 samples); *Cladosporium* (4 and 0); *Paecilomyces* (2 and 6) and *Trichoderma* (0 and 7), respectively. Iacumin et al. [18] investigated the presence of ochratoxin producing fungi on the surface of sausages from northern Italy and revealed that the most frequently species were *Penicillium nalgiovense, P. oxalicum, P. olsonii, P. chrysogenum, P. verrucosum, P. viridicatum*, *Eurotium amstelodami* and *Eupenicillium crustaceum.* Sonjak et al. [19] found that, the predominant filamentous fungal genera isolated were *Penicillium. Eurotium* spp., *Aspergillus versicolor* and *Cladosporium* spp. were isolated from meat products. Eight *Penicillium* species were identified of which *Penicillium nordicum* was recovered frequently while other penicillia were recovered less frequently.

Also, other genera were isolated in rare frequency and these were *Absidia, Cochliobolus, Emericella* (each represented by 2 spp.), *Actinomucor, Cephaliophora, Fusarium, Geotrichum, Phoma, Rhizomucor, Rhizopus, Scopulariopsis,*

*Syncephalastrum*, *Trichoderma*, *Tricothecsium* (1 sp. each) and sterile mycelia. Ismail and Zaky [11] found that the most frequently encountered fungi from luncheon meat were: *Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Mucor circinelloides*, whereas *Cladosporium sphaerospermum*, *Alternaria alternata*, *Mycosphaerella tassiana*, *P. aurantiogriseum* and *P. oxalicum* were less common. Youssef et al. [41] noticed that *Aspergillus, Penicillium, Cladosporium, Mucor, Scopulariopsis, Candida* and *Rhodotorula* were the most common fungal genera contaminating ground beef. On the other hands, some species were isolated at 7ºC but not at 28ºC and vice versa (Table 1).

**Table 1.** Total counts (TC, calculated/450 grams in all samples), number of cases of isolation (NCI, out of 30 samples) and occurrence remarks (OR) of fungal genera and species recovered from fresh beef meat on Czapek's agar at 7 and 28±2ºC.

| Genera & species | 7 ± 2ºC | | 28 ± 2ºC | |
|---|---|---|---|---|
| | TC | NCI & OR | TC | NCI & OR |
| *Absidia* | **3** | **2R** | **6** | **2R** |
| *A. corymbifera* | **3** | **2R** | **1** | **1R** |
| *A. glauca* | **0** | **0** | **5** | **1R** |
| *Actinomucor elegans* | **0** | **0** | **2** | **1R** |
| *Alternaria* | **19** | **6L** | **3** | **2R** |
| *A. alternata* | **15** | **4L** | **3** | **2R** |
| *A. chlamydospora* | **4** | **2R** | **0** | **0** |
| *Aspergillus* | **31** | **9M** | **202** | **27H** |
| *A. aculeatus* | **0** | **0** | **4** | **3R** |
| *A. awamori* | **0** | **0** | **2** | **2R** |
| *A. candidus* | **3** | **3R** | **2** | **1R** |
| *A. cervinus* | **0** | **0** | **1** | **1R** |
| *A. flavipes* | **0** | **0** | **1** | **1R** |
| *A. flavus* | **5** | **3R** | **23** | **13M** |
| *A. foetidus* | **1** | **1R** | **35** | **8L** |
| *A. fumigatus* | **0** | **0** | **22** | **8L** |
| *A. japonicas* | **0** | **0** | **1** | **1R** |
| *A. niger* | **5** | **4L** | **37** | **10M** |
| *A. ochraceus* | **0** | **0** | **1** | **1R** |
| *A. oryzae* | **0** | **0** | **9** | **5L** |
| *A. parasiticus* | **0** | **0** | **7** | **3R** |
| *A. sulphureus* | **0** | **0** | **3** | **1R** |
| *A. sydowii* | **11** | **1R** | **0** | **0** |
| *A. tamarii* | **0** | **0** | **16** | **4L** |
| *A. terreus* | **0** | **0** | **27** | **9M** |
| *A. tubingensis* | **0** | **0** | **8** | **5L** |
| *A. versicolor* | **0** | **0** | **3** | **1R** |
| *A. wentii* | **6** | **1R** | **0** | **0** |
| *Cephaliophora tropica* | **0** | **0** | **1** | **1R** |
| *Cladosporium* | 7 | 4L | 0 | 0 |
| *C. cladosporioides* | 1 | 1R | 0 | 0 |

| Genera & species | 7 ± 2ºC | | 28 ± 2ºC | |
|---|---|---|---|---|
| | TC | NCI & OR | TC | NCI & OR |
| *C. herbarum* | 4 | 2R | 0 | 0 |
| *C. macrocarpum* | 2 | 2R | 0 | 0 |
| *Cochliobolus* | 6 | 3R | 0 | 0 |
| *C. geniculatus* | 2 | 2R | 0 | 0 |
| *C. ovoidea* | 4 | 1R | 0 | 0 |
| *Emericella* | 0 | 0 | 4 | 3R |
| *E. nidulans* | 0 | 0 | 3 | 2R |
| *E. violacea* | 0 | 0 | 1 | 1R |
| *Fusarium* | 3 | 2R | 6 | 1R |
| *F. oxysporum* | 2 | 1R | 0 | 0 |
| *F. poae* | 1 | 1R | 6 | 1R |
| *Geotrichum candidum* | 0 | 0 | 6 | 2R |
| *Mucor* | 79 | 19H | 79 | 18H |
| *M. circinelloides* | 55 | 14M | 72 | 16H |
| *M. fuscus* | 0 | 0 | 3 | 1R |
| *M. hiemalis* | 15 | 5L | 4 | 2R |
| *M. strictus* | 7 | 1R | 0 | 0 |
| *M. racemosus* | 2 | 1R | 0 | 0 |
| *Paecilomyces* | 8 | 2R | 18 | 6L |
| *P. lilacinus* | 0 | 0 | 3 | 2R |
| *P. variotii* | 8 | 2R | 15 | 4L |
| *Penicillium* | 61 | 16H | 29 | 11M |
| *P. aurantiovirens* | 1 | 1R | 0 | 0 |
| *P. brevicompactum* | 11 | 1R | 0 | 0 |
| *P. canescens* | 0 | 0 | 1 | 1R |
| *P. caseicolum* | 1 | 1R | 1 | 1R |
| *P. chrysogenum* | 9 | 4L | 1 | 1R |
| *P. citrinum* | 10 | 2R | 1 | 1R |
| *P. corylophilum* | 3 | 2R | 6 | 2R |
| *P. expansum* | 1 | 1R | 0 | 0 |
| *P. glabrum* | 2 | 1R | 2 | 1R |
| *P. jenseni* | 5 | 2R | 5 | 3R |
| *P. megasporum* | 0 | 0 | 1 | 1R |
| *P. oxalicum* | 2 | 1R | 0 | 0 |
| *P. raistricki* | 0 | 0 | 4 | 1R |
| *P. purpurogenum* | 0 | 0 | 6 | 2R |
| *P. steckii* | 16 | 3R | 1 | 1R |
| *Phoma* | 12 | 2R | 4 | 1R |
| *P. exigua* | 5 | 1R | 0 | 0 |
| *P. glomerata* | 3 | 1R | 0 | 0 |
| *P. herbarum* | 4 | 2R | 4 | 1R |
| *Rhizomucor pusillus* | 0 | 0 | 8 | 2R |

| Genera & species | 7 ± 2ºC | | 28 ± 2ºC | |
|---|---|---|---|---|
| | TC | NCI & OR | TC | NCI & OR |
| *Rhizopus stolonifer* | 1 | 1R | 5 | 2R |
| *Scopulariopsis candida* | 0 | 0 | 1 | 1R |
| *Syncephalastrum racemosum* | 0 | 0 | 3 | 1R |
| *Trichoderma hamatum* | 0 | 0 | 23 | 7L |
| *Trichothecium roseum* | 0 | 0 | 2 | 1R |
| Sterile mycelia | 4 | 2R | 3 | 2R |
| Yeasts | 297 | 26R | 158 | 20H |
| Total count | 234 | | 400 | |
| No. of genera = 20 | 11 | | 17 | |
| No. of species = 67 | 37 | | 51 | |

OR = Occurrence remarks, H = High occurrence 16-30 samples, M = Moderate occurrence, 9-15 samples, L = Low occurrence, 4-8 samples, R = Rare occurrence, 1-3 samples.

The current results are greatly similar with those obtained by Tawakkol and Khafaga [42] who reported that the most commonly isolated fungi from meat were species of *Aspergillus, Penicillium, Candida* and *Rhodotorula* with *Aspergillus niger* was the most common, followed by *A. flavus, A. fumigatus* and *A. terreus. Penicillium chrysogenum, P. expansum, P. oxalicum* and *P. citrinum* were the common *Penicillium* species. Also, species of *Aspergillus, Eurotium, Penicillium, Alternaria, Emericella, Mucor, Cladosporium, Rhizopus, Botrytis, Epicoccum, Phaeacremonium* and *Phoma* were the most common in meat products such as ham [16] dry-cured mea [42], beef luncheon meat [11, 17] and fermented sausage or liver pane [36].

Battilani et al. [43] studied the pollution of dry-cured ham. They found that species from the genera *Aspergillus, Eurotium* and *Penicillium* are most frequently isolated from the surfaces of dry-cured meat products.

The experimental results showed that yeasts were isolated in high frequency of occurrence. They appeared in 87% and 67% of the samples contributing 55.9% and 28.3% of total fungi at 7ºC and 28ºC, respectively (Table 1). Nielsen et al. [44] showed the potential role of yeast in spoilage of five different processed meat products (bacon, ham, salami and two different liver patés) and found that yeasts were isolated, during storage and processing, meat products. However, with high number along the bacon production, but in low numbers during the production of Salami, cooked ham and liver pate, and in the final products, yeasts were detected in low numbers in very few samples.

### 3.1. Protease enzymes

The ability of common fungal isolates, recovered in the current study for protease enzyme was assessed. The results revealed that most isolates tested were able to produce protease. Among 152 isolates tested, 103 (67.8%) could produce the enzymes. From the positive isolates 1 exhibited high proteolytic, whereas 19 (18.4%) showed moderate production and 83 (80.6%) were weak producers (Table 2).

The high proteolytic isolates were related to *Alternaria alternata*, whereas the moderate isolates were related to *Aspergillus flavus, A. foetidus, A. terreus, Paecilomyces lilacinus, P. variotii, Penicillium brevicompactum, P.caseicolum, P. chrysogenum, P. citrinum, P. corylophilum, P. jenseni, P. steckii* and *Phoma herbarum*. The weak producers are related to *Absidia corymbifera, A. glauca, Alternaria chlamydospora, A. candidus, A. flavipes, A. flavus, A. foetidus, A. fumigatus, A. niger, A. oryzae, A. sulphureus, A. tamarii, A. terreus, A. tubingensis, Cladosporium cladosporioides, C. herbarum, Fusarium oxysporum, Mucor circinelloides, M. fuscus, M. hiemalis, Penicillium brevicompactum, P. caseicolum, P. corylophilum, P. expansum, P. jenseni, P. purpurogenum, P. steckii, Phoma exigua, P. herbarum, Rhizomucor pusillus, Trichoderma hamatum,* and Sterile myce-

lia. Ahmed and Abdel-Sater [45] reported that, among 73 isolates tested for proteolytic activity about 84.9% of the isolates (62 isolates) could produce protease with variable degrees. From the positive strains 30 isolates (48.4%) exhibited high protease production and these were related to *Aspergillus niger, A. flavus, A. terreus,* and *A. sydowii*. Nineteen (30.6%) isolates of the positive ones could produce enzyme moderately including *Fusarium oxysporum, A. niger, Cladosporium* and *Penicillium* species and thirteen (21%) isolates were weak producers.

El-Diasty and Salem [46] studied proteolytic fungi in some milk products and showed that most isolates of *A. flavus, A. niger, Cladosporium* spp. *Mucor* spp. and *Penicillium* have high proteolytic activity. Ghatass et al. [47] assumed that increasing permeability of the cell walls is caused by the same autolytic (enzymatic) and bacterial actions that cause deteriorations, since both give rise to the decomposition of proteins. Also, Djamel et al. [48] studied acid protease production by species of *Penicillium*. Saleem and El-Said [49] screened thirty-one fungal isolates (representing 16 genera, 28 species and 3 varieties) collected from beef luncheon meat for their abilities to produce protease and revealed that 11 isolates (35.48%) exhibited high protease production, 15 isolates (48.39%) had moderate and 5 (16.13%) were low. *Aspergillus flavus, Gibberella fujikuroi* and *Penicillium chrysogenum* were the most active producers.

### 3.2. Lipase enzymes

The ability of 152 isolates, to produce lipase were determined. The results revealed that most of isolates tested produced lipase enzymes. From the tested isolates 96 isolates (63.2%) could produce the enzyme. From the positive isolates, 3 (3.1%) exhibited high enzyme production, whereas 25 (26.1%) showed moderate production and 68 (70.8%) were weak producers (Table 2).

The results indicated that the high lipolytic producers were related to *Alternaria alternata, Aspergillus niger,* and *Paecilomyces variotii* whereas the moderate were related to *Alternaria alternata, Aspergillus awamori, A. flavus A. foetidus, A. niger, A. tubingensis, A. wentii, Cephaliophora tropica, Cladosporium herbarum, Mucor circinelloides, Pencillium chrysogenum, P. jenseni P. steckii, Phoma exigua, P. herbarum* and *Trichoderma hamatum,* while the remaining species (68 isolates) exhibited weak producers (Table 2). Nasser et al. [50] studied lipase production by 90 fungal isolates from keratinaceous materials and observed that 38% of the isolates produced this enzyme. Among the positive strains 14 isolates exhibited the highest lipase production and these were related to *Aspergillus versicolor, A. wentii, Geotrichum candidum, Penicillium camemberti, P. chrysogenum, P. jensenii, P. roqueforti, P. verrucosum* and *Scopulariopsis brevicaulis.* Twenty-four isolates could produce enzyme with moderate degree and 31 were weak. El-Diasty and Salem [46] studied lipolytic fungi in some milk products found that *Geotrichium* spp. and most isolates of *Candida lipolytica, C. parapasillosis* were lipolytic.

Aravindan et al. [51] reported that the main fungal producers of commercial lipases were *A. niger, A. terreus, A. carneus, C. cylindracea, Mucor miehei, Rhizopus arrhizus, R. delemar, R. japonicus, R. niveus* and *R. oryzae.* Saleem [17] isolated thirty one fungal species and 3 varieties from 30 samples of beef luncheon meat collected from different supermarkets in Qena. Screening of 31 isolates for their abilities to produce lipase showed that, ten isolates showed high production, while sixteen isolates were moderate and 5 isolates were low. They also found that *Aspergillus niger, Fusarium oxysporum* and *Nectria haematococca* were the highest lipase producers. Griebeler et al. [52] noticed that among 24 fungal isolates, 5 were good lipase producers and these were related to *Penicillium* and *Aspergillus* genera. Nwuche and Ogbonna [53] showed that the highest lipase producing strains belong to *Trichoderma* while the lowest was *Mucor* sp. The lipase activity of the *Aspergillus* species was high but varied significantly among the isolates which probably were different species of *Aspergillus.*

Rajendra [54] found that lipase production by seed-borne fungi was high in *Penicillium notatum* followed by *Fusarium equiseti* as compared to other fungi. While, *Curvularia lunata* and *C. pellescens* showed no lipase activity. Similar results were obtained by numerous workers [55, 56]. Also, similar results were observed by numerous workers [57-63].

**Table 2.** Protease and lipase production by fungal isolates recovered in the present investigation.

| Genera & species | NIT | Protease production | | | | Lipase production | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | NIP | High | Moderate | Weak | NIP | High | Moderate | Weak |
| *Absidia corymbifera* | 2 | 2 | — | — | 2 | 1 | — | — | 1 |
| *A. glauca* | 2 | 1 | — | — | 1 | — | — | — | — |
| *Actinomucor elegans* | 1 | — | — | — | — | — | — | — | — |
| *Alternaria alternata* | 7 | 7 | 1 | - | 6 | 6 | 1 | 1 | 4 |
| *A. chlamydospora* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |
| *Aspergillus awamori* | 1 | — | — | — | — | 1 | — | 1 | — |
| *A. candidus* | 2 | 1 | — | — | 1 | 2 | — | — | 2 |
| *A. cervinus* | 1 | — | — | — | — | — | — | — | — |
| *A. flavipes* | 1 | 1 | - | — | 1 | 1 | - | — | 1 |
| *A. flavus* | 12 | 11 | - | 3 | 8 | 7 | - | 2 | 5 |
| *A. foetidus* | 7 | 4 | - | 1 | 3 | 4 | - | 3 | 1 |
| *A. fumigatus* | 6 | 3 | - | — | 3 | 5 | - | — | 5 |
| *A. niger* | 11 | 1 | - | — | 1 | 6 | 1 | 4 | 1 |
| *A. oryzae* | 2 | 1 | - | — | 1 | 1 | - | — | 1 |
| *A. parasiticus* | 1 | - | — | — | — | — | — | — | — |
| *A. sulphureus* | 1 | 1 | - | — | 1 | - | — | — | — |
| *A. sydowii* | 1 | - | — | — | — | — | — | — | — |
| *A. tamari* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |
| *A. terreus* | 14 | 14 | — | 1 | 13 | 11 | — | — | 11 |
| *A. tubingensis* | 2 | 2 | — | — | 2 | 2 | — | 1 | 1 |
| *A. versicolor* | 1 | - | — | — | — | — | — | — | — |
| *A. wentii* | 1 | 1 | - | 1 | — | 1 | — | 1 | — |
| *Cephaliophora tropica* | 1 | - | — | — | — | 1 | — | 1 | — |
| *Cladosporium cladosporioides* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |
| *C. herbarum* | 2 | 2 | - | - | 2 | 2 | - | 2 | - |
| *C. macrocarpum* | 1 | 1 | - | 1 | - | 1 | - | - | 1 |
| *Cochliobolus geniculate* | 1 | - | - | - | - | - | - | - | - |
| *Emericella nidulans* | 1 | — | — | — | — | 1 | — | — | 1 |
| *F. oxysporum* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |
| *Mucor circinelloides* | 16 | 11 | — | — | 11 | 7 | — | 2 | 5 |
| *M. fuscus* | 3 | 3 | — | — | 3 | 3 | — | | 3 |
| *M. hiemalis* | 5 | 4 | — | — | 4 | 4 | — | — | 4 |
| *Paecilomyces lilacinus* | 2 | 2 | — | 2 | — | 2 | — | — | 2 |
| *P. variotii* | 8 | 1 | — | 1 | — | 2 | 1 | — | 1 |
| *Penicillium brevicompactum* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |
| *P. caseicolum* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |
| *P. chrysogenum* | 2 | 2 | — | 2 | — | 2 | — | 1 | 1 |
| *P. citrinum* | 1 | 1 | — | 1 | — | 1 | — | — | 1 |
| *P. corylophilum* | 2 | 2 | — | 1 | 1 | 2 | — | — | 2 |
| *P. expansum* | 1 | 1 | — | — | 1 | 1 | — | — | 1 |

| Genera & species | NIT | Protease production | | | | Lipase production | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | NIP | High | Moderate | Weak | NIP | High | Moderate | Weak |
| *P. jenseni* | 5 | 4 | – | 3 | 1 | 3 | – | 2 | 1 |
| *P. rubrum* | 1 | 1 | – | – | 1 | – | – | – | – |
| *P. steckii* | 4 | 4 | – | 1 | 3 | 3 | – | 1 | 2 |
| *Phoma exigua* | 3 | 1 | – | – | 1 | 1 | – | 1 | – |
| *P. herbarum* | 4 | 4 | – | 1 | 3 | 4 | – | 1 | 3 |
| *Rhizomucor pusillus* | 1 | 1 | – | – | 1 | – | – | – | – |
| *Trichoderma hamatum* | 4 | 1 | – | – | 1 | 1 | – | 1 | – |
| *Trichothecium roseum* | 1 | – | – | – | – | – | – | – | – |
| Sterile mycelia | 1 | 1 | – | – | 1 | 1 | – | – | 1 |
| Total isolates | 152 | 103 | 1 | 19 | 83 | 96 | 3 | 25 | 68 |

NIT = Number of isolates tested. NIP = Number of isolates positive. H = High activity, 3-2.1 cm for proteolytic, 2.4-1.7 cm for lipolytic. M = Moderate activity, 2-1.1 cm, 1.6-0.8 cm, W = Weak activity, 1-0.1 cm, 0.7-0.1 cm.

In conclusion, because worldwide population growth and globalization of the food supply, the control of meat spoilage becomes essential in order to increase its shelf life and maintain its nutritional value, texture and flavor. Proper handling, pretreatment and preservation techniques can improve the quality of meat and meat products and increase their shelf life. For controlling enzymatic, oxidative and microbial spoilage, low temperature storage and chemical techniques are the most common in the industry today. It is essential to store the meat at lower than 4°C immediately after slaughtering and during transport and storage as it is critical for meat hygiene, safety, shelf life, appearance and eating quality. Although, microbial and enzymatic spoilage can be stopped or minimized at lower temperature.

## AUTHOR'S CONTRIBUTION

All authors shared in the experimental designed and assisted in the work, formatting the tables, interpretation of data and in preparation and editing of the manuscript. The final manuscript has been approved by all authors.

## TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this article.

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