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Lipolysis Activity of Aflatoxins and Ochratoxins Producing Fungal Strains Contaminating Fresh Cow Meat

A. A. El-Shahir[1], M. A. Abdel-Sater[2] and A. S. Yassein[1]

[1]Botany and Microbiology Department, Faculty of Science, South Valley University, Qena, Egypt.
[2]Botany Department, Faculty of Science, Assiut University, Egypt.

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Fungal contamination of meat is the major cause hydrolyzation of lipids and as a result, change the meat flavor also nutrition value decreases. In this work, we tested Seventy-two species in addition 2 species varieties isolated from fresh cow meat for their ability to produce lipase enzyme. The results confirmed that all fungal isolates can produce lipase enzyme with variable degrees. The high producer's isolates were Aspergillus flavus and Scopulariopsis fusca so they were chosen for moreover studies for optimization of environmental and nutritional conditions for output of lipase. The results affirmed that ideal temperature, incubation period and pH for maximum production by the two strains were; 30°C, 6 days and pH 6 but the difference in the used carbon and nitrogen sources as follow olive oil and ammonium molybdate and sunflower oil and potassium nitrate as a sole carbon and nitrogen sources in basal medium which is initially adjusted to pH 6 for maximum lipase production. Four isolates were used as a proof for production of aflatoxins and ochratoxins by the fluorometric method the results confirmed that all the tested isolates had the ability for aflatoxins and ochratoxins production and HPLC analysis confirmed these results.

Keywords: Fresh cow meat, fungal contamination, lipase enzyme, aflatoxin, and ochratoxin

Lipases induce the hydrolysis and the creation of esters formed from glycerol and long chain fatty acids. Microbial lipases are commercially important as they are more steady if it is put in compare with plant and animal lipases and they can be got an inexpensive way. The implementations of lipases include organic synthesis, hydrolysis of fats and oils, modification of fats, flavor improvement in food processing and chemical tests (Martinelle *et al*., 1995; Sharma *et al*., 2001). When lipolytic fungi contaminating meat products, they may be responsible for the unpleasant or undesirable flavors rendering them unmarketable or even not suitable for consumption. Also, protein hydrolysis by fungi in foods may produce a variety of odor and flavor defects. Thus, selected sterilization temperature may confer microbial sterility, but not inactivate considerable portion of microbial lipase leading to later loss of product. This substantiates the hypothesis that reported by (Smith and Hass 1992). Mycotoxins are low molecular weight yields of the fungal secondary metabolite, created mainly by *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* genera. These metabolites are virulent for humans and animals if they expend or respire, and exposition to mycotoxins through the contaminated feed is one of the main hazards affecting ruminant health

*Corresponding author E-mail: dr.amanyatta@yahoo.com

(Bennet and Klich, 2003; Kalac and Woolford, 1982). The contamination of meat with mold leads either to food poisoning or spoilage and render it to be in less quality (Rieth, 1973; Mossel, 1977 and Waffia and Hassan, 2000). Also, the consuming of the contaminated meat by mold and their mycotoxins induces hemorrhages with hepatotoxic, nephrotoxic, neurotoxic, dermatotoxic, genotoxic, teratotoxic, carcinogenic or hormonal effects and immunosuppression (Cheo, 1991 , Hassan *et al*., 2004 and Abd - Elghany & Sallam 2015).

MATERIALS AND METHODS

Screening of fungal isolates for lipase production

The lipolytic action was scaled using tributyrin as a lipid substrate (Cardenas *et al*., 2001). The medium used contained (g/l) peptone, 10g; $MgSO_4.7H_2O$, 2g; $CaCl_2.2H_2O$, 0.2g; tributryin emulsion, 10 (w/v); agar, 15g with pH 6. Tributyrin was sterilized separately by autoclaving for 15 minutes at 1.5 atm. and 1 ml was added to 100 ml of the sterile basal medium before solidification. Using a sterile crock borer (10 mm diameter) inoculum discs bearing mycelia from a previously prepared agar culture were obtained. Disc in triplicates was placed in cavities made in the assay agar medium. The cultures were incubated for 48 hours at 25°C. The diameter of the clear zone around each disc was measured and the average was calculated.

Assay for lipase activity

The method described by Mateos Diaz *et al*. (2006) was employed. This method was designed to estimate the amount of free fatty acids (FFA) in m mole lose by the enzyme action. 10 ml of olive oil emulsion solution (0.1% olive oil) was diluted by 20 ml distilled water and added to 0.25 ml of sodium taurocholate and 5 ml of the crude enzyme (fungal filtrate), pH was adjusted to 8.8. After 20 minutes of incubation at 30°C with shaking, the mixture titrated with 0.05 M NaOH until pH 8.8 (the original pH). The consumed volume of NaOH was estimated and the volume activity calculated. Samples with 5 ml distilled water (replaced the enzyme solution) was used as a blank.

Factors affecting on lipase production

The effect of some ecological and nutritional factors on the production of lipase enzyme by two fungal species (*Aspergillus flavus* and *Scopulariopsis fusca*) were studied. Since these species were found to be the most active in lipase production.

Effect of temperature and time course

The inoculated flasks were incubated at 20, 30 and 40°C for 14 days and harvested at 48 hours intervals. Cultures fluids were filtered and assayed for lipase production.

Effect of pH values

The test organisms (*Aspergillus flavus* and *Scopulariopsis fusca*) were grown on the medium described earlier. The initial pH values of the medium were regulated with citrate buffer to various values from 2 to 14. Inoculated flasks were incubated for 6 days at 30°C (the best temperature and time course for lipase production), then filtered and assayed for lipase production.

Effect of carbon sources

To study the effect of oils as carbon sources on lipase production by chosen fungal species, the basal medium previously mentioned was supplemented sporadically with 0.1% emulsion of various oils and pH of the medium was adjusted to 6 (the best pH for lipase production). These oils were: sunflower oil, flax oil, olive oil, peanuts oil, hsalban oil. Inoculated flasks were incubated at 30°C for 6 days, then filtered and the supernatant was utilized for the assay of the enzyme activity.

Effect of nitrogen sources

To estimate the effect of nitrogen sources on the production of lipase enzyme the 3 g/L of sodium nitrate as inorganic nitrogen source in the basal medium was substituted by an equivalent amount of different nitrogen compounds for example yeast extract, peptone, ammonium molybdate, ammonium sulfate, and potassium nitrate as well as sodium nitrate as control. the pH of the medium was adjusted to 6. Inoculated flasks were incubated at 30°C for 6 days. After the incubation period was ended, the enzyme activity was assayed in the supernatant filtrate.

Screening of fungal isolates for mycotoxins production

1. Fungal isolates: A total of 4 isolates of prevalent fungi, from fresh cow meat belonging to *Aspergillus fumigatus*, *A. niger* and *Penicillium chrysogenum* (2 isolates) were examined for their ability for production of mycotoxins.

2. Cultivation and inoculation of fungal cultures: Sucrose yeast extract liquid medium was used for mycotoxins screening. The composition of sucrose yeast extract liquid medium was sucrose, 40 g, and yeast extract, 20 g per liter of distilled water (pH 6±0.2). Erlenmeyer flasks of 250 ml capacity were used. Each flask contained 50 ml of the desired medium. Flasks were sterilized at 1.5 atmospheres for 20 min. and inoculated after cooling with 2 discs taken from 7 day-old culture. Cultures were incubated for 15 days at 28°C (Gabal *et al*., 1994).
3. Detection of mycotoxins: The levels of mycotoxins were measured by a fluorometric method using aflatoxins or ochratoxins standards for adjustment of fluorometer before reading the toxins content of the tested samples (Hansen, 1993; VICAM, chemists. Inc., U.S. America).

RESULTS AND DISCUSSION

Lipolysis activity

Four isolates (5.4 % of total isolates) exhibited high lipolytic activity (clear zones 14-17 mm) and these isolates were: *Aspergillus fumigatus*, *A. flavus*, *Cladosporium spongiosum,* and *Scopulariopsis fusca*. Thirty-five isolates (47.3% of total isolates) were found to be of moderate lipolytic activity and the weakly lipolytic (clear zone less than 10) isolates were represented by 35 isolates (47.3% of total isolates) as shown in the table (1). These results similar to those obtained by Abdel-Rahman and Saad (1989) and Banwart (1989) found that fungi isolated from meat and meat products e.g. *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium* and other had lipolytic and proteolytic activity. Godtfredsen (1990) who reported that lipolytic microorganisms are the widest spread such as *Aspergillus niger*, *A. flavus* and *A. fumigatus* that produce lipases it is to note that, microbial lipases are often heat resistant. In Egypt, Tawakkol and Khafaga (2007) found that all the examined species of *Aspergillus* (*A. niger*, *A. flavus*, *A. ochraceus*, *A. terreus*, *A. parasiticus* and *A. candidus*), *Penicillium* (*P. chrysogenum*, *P. citrinum*, *P. expansum* and *P. oxalicum*), *Scopulariopsis* spp, *Mucor* spp, *Rhizopus* spp, *Candida albicans* and *Fusarium* spp isolated from cattle and buffalo meat had lipolytic activity. In Qena city, Saleem (2008) screened a total of thirty-one fungal species and 3 species varieties isolated from beef luncheon meat for their abilities to produce lipase enzyme. He found that ten isolates represented 32.26% of total isolates appeared high lipase production, while sixteen isolates (51.61%) were moderate and 5 isolates (16.13%) were low producers. *Aspergillus niger*, *Fusarium oxysporum,* and *Nectria haematococca* produced the highest amount of lipase enzyme so he used these isolates in further studies. The integration of five food preservatives (Disodium phosphate, sodium benzoate, citric acid, potassium sorbate, and sodium citrate) individually in the culture medium of lipase production exhibited an inhibitive effect on the mycelial growth and enzyme production by the three tested isolates. Ouf *et al*. (2010) reported that most isolates of *Aspergillus flavus*, *A. niger*, *Cladosporium* spp., *Mucor* spp. and *Penicillium* spp. Isolated from meat and meat products have the ability to produce lipase and protease enzymes.

Effect of environmental and nutritional factors on lipase production

Effect of temperature and time course

Maximum lipase production by both of *Scopulariopsis fusca* and *Aspergillus flavus* was achieved 6 days after incubation at 30°C. Cultures incubated at 40°C produced the lowest quantity of the enzyme whereas those incubated at 20°C yielded moderate amounts of lipase enzyme (Figs. 1 and 2).

Effect of pH values

The data represented in Figs. (1 and 2) shows that considerable amounts of lipase enzyme were detected by *Scopulariopsis fusca* and *Aspergillus flavus* if the culture medium was initially adjusted at a pH range of 4-8 and the maximum production could be achieved at pH 6. Synthesis of lipase enzyme was greatly inhibited at pH lower than 4 or higher than 10.

Effect of different carbon sources

Among the 5 carbon sources incorporated separately in culture medium olive oil and sunflower oil yielded the highest amounts of lipase enzyme by *Scopulariopsis fusca* and *Aspergillus flavus*, respectively. On the other hand considerable amounts of lipase enzyme were produced in case of using hsalban oil and equal amounts of lipase enzyme were produced in case of using sunflower oil, flax oil and peanuts oil by *Scopulariopsis fusca* but in case of *Aspergillus flavus*; moderate amounts of lipase enzyme were produced by using hsalban

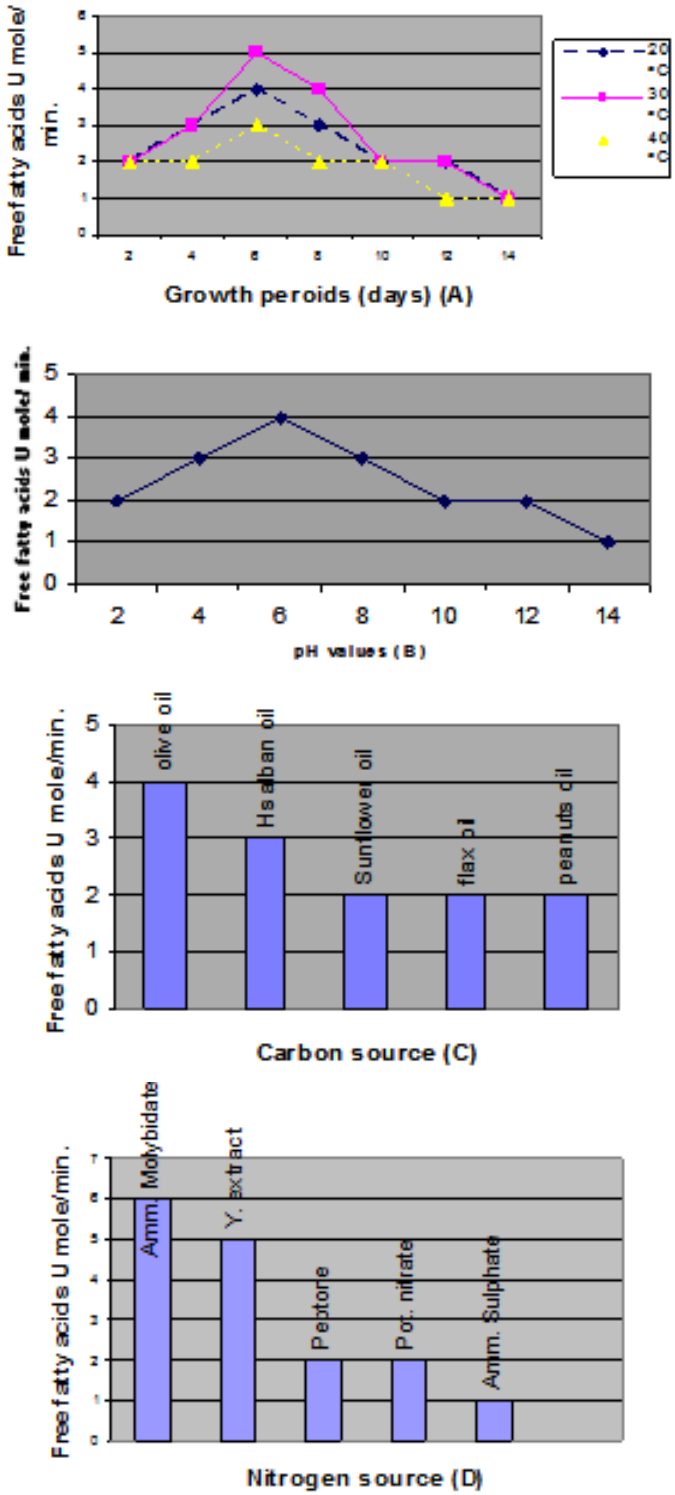

Fig. 1. Effect of time course and temperature (A), pH values (B), different carbon sources (C) and different nitrogen sources (D) on the production of lipase enzyme by *Scopulariopsis fusca*

Table 1. Degree of lipolytic activity (calculated as the average diameter of the clear zone in mm) of the tested fungal isolates

Fungal Isolates	The diameter of the clear zone (mm)
Acremonium cerealis	11.5 M
A. kiliense	10 W
A. rutilum	13 M
A. strictum	11 M
Alternaria alternate	10 W
Aspergillus clavatus	12 M
A. flavo-furcatis	12 M
A. flavus	16 H
A. fumigatus	15 H
A. janus var. *brevis*	11 M
A. niger	10 W
A. terreus var. *aureus*	12 M
A. versicolor	10 W
Bahusakala olivaceonigra	12 M
Botryotrichum atrogriseum	11 M
B. piluliferum	10 W
Circinella muscae	10 W
Cladosporium chlorocephalum	11 M
C. cladosporioides	10 W
C. cucumerinum	11 M
C. diaphanum	10 W
C. spongiosum	14 H
C. sphaerospermum	10 W
C. tenuissum	13 M
C.uredinicola	10 W
C.variable	11 M
Cochliobolus carbonus	11 M
C. spicifer	10 W
Dreschslera fugax	10 W
D. monoceras	11 M
Emericella nidulans	10 W
Epicoccum purpurascens	10 W
Eurotium chevalieri	10 W
Gibberella fujikuroi	12 M
Memnoniella subsimplex	10 W
Mucor circinelloides	10 W
M. hiemalis	10 W
M. racemosus	11 M
Mycosphaerella tassiana	10 W
Myrothecium cinctum	10 W
M. verrucaria	10 W
Nectria bacterioides	10 W
N. haematococca	10 W
N. viridescens	10 W
Neurospora crassa	10 W
Paecilomyces carenus	11 M
P. lilacinus	12 M
P. varotii	10 W
Papulospora immerse	10 W
Phaeoisaria clavulata	13 M
Penicillium aurantiogriseum	11.5 M
P. chrysogenum	12.5 M
P. corylophilum	12 M
P. duclauxii	11 M
P. funiculosum	11 M
P. oxalicum	11.5 M
P. purpurogenum	11 M
P. steckii	11 M
P. variabile	13 M
P. waksmanii	10 W
Phoma exigua	10 W
P. levelii	10 W
P. medicaginis	12 M
Plectosphaerella cucumerina	12 M
Pleospora herbarum	12 M
Scopulariopsis brevicualis	10 W
S. fusca	17 H
Scytlidium lignicola	11.3 M
Stachybotrys state of *melanopasamma pomiformis*	10 W
Stemphylium sarciform	10 W
Syncephlastrum racemosum	10 W
Ulocladium atrum	12 M
U. botrytis	10 W
U. tuberculatum	11 M

*Activity Remarks: High activity, H= from 14 – 17 mm; Moderate activity, M= from 11 - 13 mm; Weak activity, W= less than 10 mm.

oil, flax oil, peanuts oil and olive oil (Figs. 1 and 2).

Effect of different nitrogen sources

As presented in Figs. (1 and 2) ammonium molybdate and potassium nitrate were the best nitrogen sources required to induce the highest yield of lipase enzyme by *Scopulariopsis fusca* and *Aspergillus flavus*, respectively. In case of *Scopulariopsis fusca* moderate amount of lipase enzyme was produced by the incorporation of yeast extract and similar amounts were produced by using peptone and potassium nitrate and the lowest amount of lipase enzyme was yielded by using ammonium sulfate. On the other hand, the moderate amount of lipase enzyme was produced by *Aspergillus flavus* in the presence of ammonium molybdate and equal amounts were produced by using ammonium sulfate, peptone and yeast extract.

These findings are almost in agreement with those reported by Rapp (1995) found that

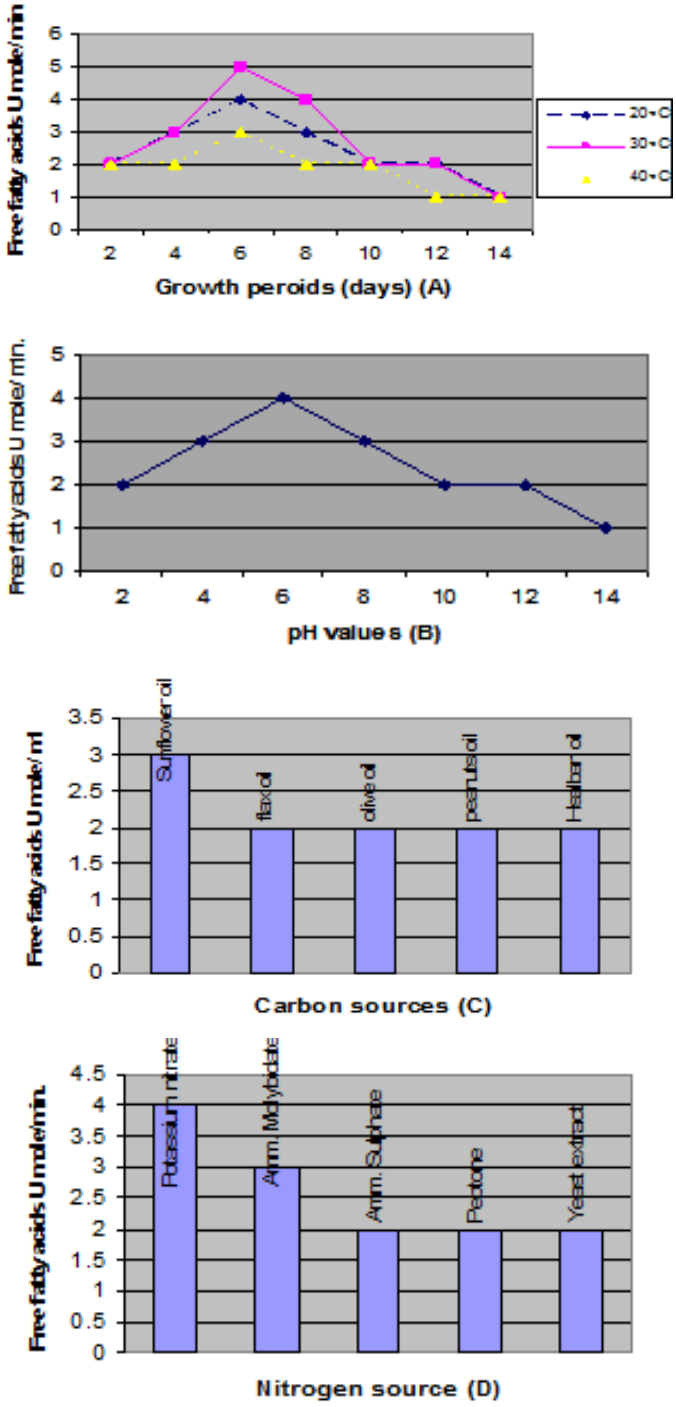

Fig. 2. Effect of time course and temperature (A), pH values (B), different carbon sources (C) and different nitrogen sources (D) on the production of lipase enzyme by *Aspergillus flavus*

the maximum production of lipase enzyme by *Fusarium oxysporum* f. sp. *vasinfectum* required peptone. Additions of trimyristin, olive oil, span 85, and oleic acid to growing shake-flask cultures, and the addition of oleic acid to suspensions of washed mycelium. Synthesis of lipase activity was repressed by glucose and glycerol. Lipase activity was inhibited by oleic acid and its sodium salt, but not by glycerol. The lipase activity remained constant during 1h incubation at pH 4 to 10.5 and 30°C. It was stable in 25% (v/v) acetone, ethanol, and n-propanol and was catalyzed remarkably in n-hexane. Kamini *et al*., (2000) inspected a number of factors affecting the production of extracellular lipase by *Cryptococcus* sp. S-2. Sequential optimization of nitrogen, carbon sources and inducers promoted lipase activity and under optimum conditions, the lipase activity was 65.7 U/ml of the culture medium in 120 h at 25°C and at pH 5.6. Sardine oil, soybean oil, and triolein oil were active inducers of lipase production. Abbas *et al*. (2002) isolated *Mucor* sp strain during a screening of lipolytic fungus which may play a role in the acidification of palm oil. He found that the highest lipase production amounting to 57 U/ml when culture conditions were optimized was achieved after 6 days of cultivation. Ul-Haq *et al*. (2002) screened ten mold cultures for the production of lipases. He found that *Rhizopus oligosporous* GCBR-3 supported maximum production of lipase (48.0U/g substrate) under optimum environmental and cultural conditions such as wheat bran/flask and incubation at 30°C for 48 h. Tan *et al*. (2004) optimized the culture medium including nitrogen source, carbon source, and metal ions, for lipase production from *Penicillium camembertii* Thom

Table 2. Mycotoxigencity of the tested fungal isolates

Types of examined isolate	Average total count	Sample No.	Type of toxin	Levels of toxin (PPB)
Aspergillus fumigatus	240	40	Aflatoxin	3
			Ochratoxin	7.2
Aspergillus niger	235	25	Aflatoxin	3.3
			Ochratoxin	2
Penicillium chrysogenum (1)	355	7	Aflatoxin	4.6
			Ochratoxin	3.2
Penicillium chrysogenum (2)	260	6	Aflatoxin	2.8
			Ochratoxin	3.1

Table 3. HPLC analysis of aflatoxins and ochratoxins produced by *Aspergillus fumigatus*.

Analyte 8 Parameter	Test Method	Description
Aflatoxin B_1	ACAL-APR-79-00	% of total toxins: 14.806
Aflatoxin G_1	ACAL-APR-79-00	% of total toxins: 33.918
Aflatoxin G_2	ACAL-APR-79-00	% of total toxins: 0.291
Ochratoxin A	ACAL-APR-79-00	% of total toxins: 50.985

Table 4. HPLC analysis of aflatoxins and ochratoxins produced by *Penicillium chrysogenum* (1)

Analyte 8 Parameter	Test Method	Description
Aflatoxin B_1	ACAL-APR-79-00	% of total toxins: 75.501
Aflatoxin B_2	ACAL-APR-79-00	% of total toxins: 8.465
Aflatoxin G_2	ACAL-APR-79-00	% of total toxins: 0.387
Ochratoxin A	ACAL-APR-79-00	% of total toxins: 15.647

PG-3 and the optimal medium consisted of soybean meal (fat free) 4%, Jojoba oil 0.5%, $(NH_4)_2HPO_4$, 0.1% Tween 60, initial pH 6.4 and the inoculation was at 28°C for 96 h. The optimal pH and temperature for lipase activity were 6.4 and 48°C, respectively. Liu *et al*. (2009) studied the effects of temperature, pH, and various components in detergent on the activity and stability of a lipase produced by a soda lake fungus strain *Fusarium solani* N4-2. The lipase showed the maximal activity at pH 9.0 in a glycine-NaOH buffer at 30°C. At 0°C and 10°C, 52.3 % and 82.6% its maximum activities were detained, respectively.

The occurrence of mycotoxins in the culture of most common fungi

The results in table (2) revealed that the four tested isolates had ability for aflatoxins and ochratoxins production and the results were confirmed by HPLC analysis as shown in tables (3, 4, 5 and 6) and these results in agreement with Glinsukon *et al*. (1979) found that *A. niger* produces low level of aflatoxin. Mahmoud *et al*. (2001) found that many of isolated fungi from local meat products (luncheon and minced meat) and livers of poultry and imported bulls have mycotoxin producing potential. Results of mycotoxins analysis revealed that 45 % of the examined samples were positive. Aflatoxins (B1, B2, G1, and G2), ochratoxin A, citrinin and sterigmatocystin were detected. Samples of livers imported bulls contained the highest level of aflatoxins and ochratoxin A. Varga *et al*. (2001) reported that *Aspergillus auricomus*, *A. fumigatus*, *A. glaucus*, *A. melleus*, *A. ostianus*, *Penicillium aurantiogriseum*, *P. canescens*, *P. chrysogenum*, *P. commune* and others had ability to produce ochratoxin A. Czerwiecki *et al*. (2002 a, b) found that *Penicillium chrysogenum*, *P. cyclopium*, *P. griseofulvum*, *P. oslitum*, *Aspergillus flavus*, *A. versicolor* and *Eurotium glaucum* were ochratoxin A producers. Frisvad *et al*. (2006) found that *Aspergillus flavo-fucus*, *A. glaucus*, *A. niger*, *A. oryzae*, *A. ostianus*, *A. sulphureus*, *A. tamarii*, *Penicillium citrinum*, *P. citromyces*, *P. digitatum*, *P. frequentans*, *P. expansum*, *P. puberulum,* and others could produce aflatoxins. Tawakkol and Khafaga (2007) tested 27 strains of *Aspergillus flavus* isolated from 200 samples of meat and 120 environmental samples for the production of aflatoxins, 21 isolates (77.7%) were found toxigenic. They could produce aflatoxins B1, B2 or G1 either singly or combined. Also, they found that 15 out of 26 isolates of *Aspergillus ochraceus* produced ochratoxins. Abdel- Kareem (2010) revealed that four isolates out of nine isolates of *A. niger* and 3 isolates of *A. ochraceus* could produce ochratoxin A. De Curtis *et al.* (2012) reported ochratoxin A production is mainly associated with *Aspergillus carbonrius* and *A. niger* or *nigris* section species. Abd - Elghany &

Table 5. HPLC analysis of aflatoxins and ochratoxins produced by *Penicillium chrysogenum* (2)

Analyte Parameter	Test Method	Description
Aflatoxin B_1	ACAL-APR-79-00	% of total toxins: 67.111
Aflatoxin B_2	ACAL-APR-79-00	% of total toxins: 6.117
Aflatoxin G_1	ACAL-APR-79-00	% of total toxins: 0.466
Aflatoxin G_2	ACAL-APR-79-00	% of total toxins: 0.243
Ochratoxin A	ACAL-APR-79-00	% of total toxins: 26.063

Table 6. HPLC analysis of aflatoxins and ochratoxins produced by *Aspergillus niger*

Analyte Parameter	Test Method	Description
Aflatoxin B_1	ACAL-APR-79-00	% of total toxins: 64.857
Aflatoxin B_2	ACAL-APR-79-00	% of total toxins: 7.069
Aflatoxin G_1	ACAL-APR-79-00	% of total toxins: 2.165
Ochratoxin A	ACAL-APR-79-00	% of total toxins: 25.909

Sallam (2015) studied total aflatoxins (AFT) and ochratoxin A (OTA) levels were evaluated using the VICAM AflaTest and OchraTest immunoaffinity fluorometric method in a total of 50 meat products (25 each of beef luncheon and 25 beef burger) bought from different supermarkets in Mansoura city, Egypt. All the meat samples analyzed were contaminated with both AFT and OTA with mean values of 1.1 µg/kg and 5.23 µg/kg, respectively, for beef luncheon and mean values of 3.22 µg/kg and 4.55 µg/kg, respectively, for the beef burger.

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CONCLUSION

The results confirmed that all the fungal isolates can produce lipase enzyme with variable degrees. The high producer's isolates were *Aspergillus flavus* and *Scopulariopsis fusca* so they were chosen for moreover studies for optimization of environmental and nutritional conditions for output of lipase. The results affirmed that ideal temperature, incubation period and pH for maximum production by the two strains were; 30°C, 6 days and pH 6 but the difference in the used carbon and nitrogen sources as follow olive oil and ammonium molybdate and sunflower oil and potassium nitrate as a sole carbon and nitrogen sources in basal medium which is initially adjusted to pH 6 for maximum lipase production by *Scopulariopsis fusca* and *Aspergillus flavus*, respectively. Four isolates were used as a proof for production of aflatoxins and ochratoxins by the fluorometric method the results confirmed that all the tested isolates had the ability for aflatoxins and ochratoxins production and HPLC analysis confirmed these results.

REFERENCES

1. Martinelle M., Holmquist M. and Hult K. On the interfacial activation of Candida Antarctica lipase A and B as compared with Humicola lanuginose lipase. *Biochim. Biophys. Acta.*1995; **1258**: 272-276.
2. Sharma R., Chisti Y. and Banerjee U. C. Production, purification, characterizations, and applications of lipases. *Biotechnol. Advan*. 2001; **19**: 627-662.
3. Smith, J. L., and Haas, M. J. Lipolytic microorganisms. Chapter 11, Pp.183. In: APHA Compendium of Methods for the Microbiological Examination of Food. 3rd ED. Washington, USA.1992
4. Bennett, J. W., and Klich, M. Mycotoxins. *Clinical Microbiological Reviewers,* 2003; **16** (3): 497-516.
5. Kalac, P. and Woolford, M. K. A review of some aspects of possible associations between the feeding of silage and animal health. *The British Veterinary Journal*, 1982; **138**: 305-320.
6. Rieth, H. Human pathogen. Hefen und Schimmelpilze in Lebens- Futtermitteln. LH, 1973; **1**: 41-48.
7. Mossel, A. A. D. Microbiology of foods. Univ. Utrecht., Fac. Vet. Med., Netherland. 1977.
8. Wafia, H. A. and Hassan, A. A. (2000): Sanitary status of some ready to eat meat meals in Cairo and Giza Governorates. *J. Egypt. Vet. Med. Assoc*., 2000; **60**(7): 95-104.
9. Cheo, F. S. Mycotoxins food contamination mechanism, carcinogenic potential, and preventive measures. *Mutat. Res*., 1991; **259**(3): 261.
10. Hassan, A. A., Ragheb, R. R. and Rahmy, N. A. Pathological changes in cows spontaneously fed on some mycotoxins. Egypt. *Journal Comp. Path. & Clinic. Path.,* 2004; **17**(1): 282-293.
11. Abd - Elghany & Sallam Rapid determination of total aflatoxins and ochratoxins A in meat products by immuno-affinity fluorimetry. *J.Food Chem*. 2015; **6**: 179-253
12. Cardenas, J., AlVarez, E., De Castro-AlVarez, M. S., Sanchez-Montero, J. V. Screening and catalytic activity in organic synthesis of novel fungal and yeast lipases. *J. Mol. Catal. B: Enzym.* 2001; **14**: 111-123.
13. Mateos Diaz, J. C., Rodriguez, J. A., Roussos, S., Cordova, J., Abousalham, A., Carriere, F. and Baratti, J. Lipase from the thermotolerant fungus *Rhizopus homothallicus* is more thermostable when produced using solid-state fermentation than liquid fermentation procedures. *Enzyme and Microbial Technology,* 2006; **39**: 1042-1050.
14. Gabal, M. A., Hegazy, S. M. and Nagwa, Y. H. Aflatoxin production by *Aspergillus flavus* field isolates. *Vet. Human Toxicology,* 1994; **36**: 519-521.
15. Hansen, T. J. Quantitative testing for mycotoxins (VICAM) *Am. Ass. Of cereal Chemist. Inc*., 1993; **38**: 5-8.
16. Abdel- Rahman, A. H., and Saad, S. M. Studies

on the proteolytic activity of some prevalent mold species in relation to meat hygiene. *Zagazig Veterinary Medical Journal*, 1989; **17**: 228-236.

17. Banwart, G. J. Basic Food Microbiology 2nd Ed. An Avi book published by Van Nostrand Rein holds New York. 1989; 781 pages.
18. Godtfredsen, S. E. Microbial lipases. In: Microbial Enzymes and Biotechnology. 2nd Ed. W. M. Fogarty and C. T. Kelly, eds. Elsevier, London. 1990; Pp. 255.
19. Tawakkol, W. and Khafaga, N. I. Fungal contamination of meat and its environment with Special reference to the strains producing aflatoxins, ochratoxins, proteinase, and lipase enzymes. *N. Egypt. J. Microbiol.* 2007; **17**.
20. Saleem, A. Effect of some food preservatives on the lipolytic activity of beef luncheon fungi. *Mycobiology*. 2008; **36**: 167-172.
21. Ouf, J. M., Khafaga, N. I. M. and Shabana, E. S. E. Incidence of proteolytic and lipolytic molds and yeasts in some ready to eat meat products. *Assiut Veterinary Medical Journal*. 2010; **56**: 132-143.
22. Rapp, P. Production, regulation, and some properties of lipase activity from *Fusarium oxysporum* f. sp. *vasinfectum*. *Enzyme and Microbial Technology.* 1995; **17**: 832-838.
23. Kamini, N. R., Fujii, T., Kurosu, T., and Iefuji, H. Production, purification, and characterization of an extracellular lipase from the yeast, Cryptococcus sp. S-2. *Process Biochemistry.* 2000; **36**: 317-324.
24. Abbas, H., Hiol, A., Deyris, V. and Comeau, L. Isolation and characterization of an extracellular lipase from *Mucor* sp. strain isolated from palm fruit. *Enzyme and Microbial Technology.* 2002; **31**: 968-975.
25. Ul-Haq, I., Idrees, S. and Rajoka, M. I. Production of lipases by Rhizopus oligosporus by solid-state fermentation. *Process Biochemistry*. 2002; **37**: 637-641.
26. Tan, T., Zhang, M., Xu, J. and Zhang, J. Optimization of culture conditions and properties of lipase from *Penicillium camembertii* Thom PG-3. *Process Biochemistry*. 2004; **39**: 1495-1502.
27. Liu, R., Jiang, X., Mou, H., Guan, H., Hwang, H. M. and Li, X. A novel low-temperature resistant alkaline lipase from a Soda lake fungus strain *Fusarium solani* N4-2 for detergent formulation. *Biochemical Engineering Journal*. 2009; **46**: 265-270.
28. Glinsukon, T., Stitmunnaithum, V., Toskulkao, C., Buranawuti, T. and Tangkrisanavinot, V. Short communications. Acute Toxicity of Capsaicin in several animal species. *Toxicon*. 1979; **18**: 219-220.
29. Mahmoud, A. L. E., Sayed, A. M., and Abou El-Alla, A. A. Mycoflora and natural occurrence of mycotoxins in some meat products and livers of poultry and imported bulls. *Pakistan Journal of Biological Science.* 2001; **4**(5): 611-613.
30. Varga, J., Rigo, K., Reren, J. and Mesterhazy, A. Recent advances in ochratoxin research. I. Production, detection, and occurrence of ochratoxins, *Cereal Res. Commun*. 2001; **29**: 85-92.
31. Czerwiecki, L., Czajkowska, D. and Witkowska-Gwiazdowska, A. On ochratoxin A and fungal flora in polish cereals from conventional and ecological farms. Part 1: Occurrence of ochratoxin A and fungi in cereals in 1997. *Food Addit. Contam.* 2002 a; **19**: 470-477.
32. Czerwiecki, L., Czajkowska, D. and Witkowska-Gwiazdowska, A. On ochratoxin A and fungal flora in polish cereals from conventional and ecological farms. Part 2: Occurrence of ochratoxin A and fungi in cereals in 1998. *Food Addit. Contam.* 2002 b; **19**: 1051-1057.
33. Frisvad, J. C., Thrane, U., Samson, R. A. and Pitt, J. I. (Important mycotoxins, and fungi which produce them, in Advances in Food Mycology, A. D. Hocking, J. I. Pitt, R. A. Samson, and U. Thrane, eds, Springer, New York, 2006; pp. 3-25.
34. Abdel-Kareem, M. M. Microbiota and mycotoxins occurrence in some Egyptian fruit juices. M. Sc. Thesis, Botany Department, Faculty of Science, Sohag University, Sohag, Egypt. 2010
35. De Curtis F., De Felice D. V., Laniri G., De Cicco V. and Castoria R. Environmental factors affect the activity of biocontrol agents against ochratoxigenic Aspergillus carbonarius on wine grape. *International Journal of Food Microbiology,* 2012; **159**: 17-24.