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Mycological assessment of marketed duck meat in El-Qalyubia governorate markets

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ABSTRACT

This study was conducted to evaluate the mycological contamination of duck carcasses, and its hazards on public health. A total of two hundred meat samples of duck meat were taken from chilled and frozen breast and thigh (100 of each) that were collected from supermarkets in El-Qalyubia governorate for mycological examination. The mean values of yeast and mold count of the examined chilled samples were $3.1\times10^2\pm0.02\times10$ cfu/g and $5.5\times10^2\pm0.4\times10$ cfu/g in breast and thigh, respectively. Also, those frozen samples had mean values of$3.0\times10^2\pm0.3\times10$ cfu/g and $6.0\times10^2\pm0.4\times10$ cfu/g for breast and thigh, respectively. PCR amplification was resulted in three toxigenic strains of *A. flavus, A. fumigatus and A. niger* were isolated from examined samples. Aflatoxin residues were detected in breast and thigh of 0.26 and 0.33 ng/g. Thus, strict hygienic precautions during processing of duck products should be adopted to reduce mold contamination and mycotoxin production.

1. INTRODUCTION

Due to the high need of the world to animal protein which is considered the most important element than other food elements at all, because it contributes in all building processes which are responsible for repairing of the damaged body tissues. So, the world as a whole begins to increase poultry production, processing and utilization. Duck is still very popular and in strong demand in many area of the world, especially in Asia. In addition, Duck and geese production accounts for about 7.5% of the total world poultry meat production (Pigel, 2004).

Duck meat considered a good source of protein for humans(Adzitey et al., 2012a) and is high in iron, selenium, and niacin, as well as containing fewer calories than many cuts of beef (Adzitey et al., 2012b).

Mold and yeast comprise a large group of microorganisms which are ubiquitous in nature due to easy dissemination and their vegetative spores, which are produced in large numbers and can present in the environment for a long period. Contamination of duck meat with fungi starts in the environment of the slaughter halls due to a lack of hygienic measures through air, wall, floor, utensils, feather and intestinal contents of the slaughtered birds (Mansour, 1986).Contaminated feed is a main source for mold and mycotoxin infection of farm animal (Sayedetal., 2000). A long with molds, yeasts belong to the class mycotaor fungi, they are microscopic, single-celled organisms generally larger than the bacteria.

Fungi are not only major spoilage agents of meat results in a reduction of quality with significant economic losses but also cause contamination of meat with secondary metabolites called mycotoxins.

The most well-known among the mycotoxins are aflatoxins (AFs), which are a group of heterocyclic metabolites produced by the fungi of the genus AspergillusThe four naturally occurring AFs: aflatoxins B1, B2, G1 and G2, are toxic, mutagenic and carcinogenic compounds (CAST, 2003), and having teratogenic, hepatotoxic, mutagenic and teratogenic effects(Kensleretal.,2011). A potential immunosuppressant and nutritional interference effect has also been reported (Williams *et al.*, 2004).

Thus, this study was designed to investigate the mycological state marketed duck meat in El-Qalyubia governorate markets.

2. MATERIAL AND METHODS

2.1. Collection of samples:

Two hundred random samples of chilled and frozen duck meat with skin (breast and thigh (fifty of each) were collected from different localities in Benha city in El-Qalyubia governorate markets, Egypt in winter season. Samples were identified, packed and transferred to the laboratory in icebox under complete aseptic conditions without undue delay and subjected to the mycological examination.

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2.2. Preparation of samples (APHA, 2001) and investigation Twenty-five grams of examined duck meat samples were aseptically excised and homogenized in 225 ml of sterile buffered peptone water 0.1% at 2000 rpm for 1-2 min using a sterile homogenizer. Such homogenate represents the dilution of 10-1, and then decimal dilutions were done. Then prepared samples subjected to the following examination:
2.2.1. Mold and yeast count (Bailey and Scott, 1998)
2.2.2. Isolation and identification of isolated mold and yeast based on their micromorphological properties (Pitt and Hocking 2009)
2.2.3.Identification of toxigenic mold strains by using PCR
2. 2.4. Determination of aflatoxin residues by LC-MS/MS

3. RESULTS

It is evident from the result recorded in table (1) that the mean value of yeast and mold counts (cfu/g) in the examined chilled duck meat were 3.1×10^2 $\pm0.02\times10^2$ and $5.5\times10^2\pm0.4\times10^2$and also, in frozen ones were $3.0\times10^2\pm0.3\times10^2$and $6.0\times10^2\pm0.4\times10^2$in breast and thigh samples, respectively. In comparing chilled and frozen samples, no difference was found neither between the breast samples nor the thigh samples. While, a significant ($p\leq0.05$) difference has been found between breast and thigh samples Moreover, results in table (2) revealed that the percentage of accepted samples for mold and yeast count were 80% , 72%, 36% and 24% in chilled and frozen breast and thigh, respectively, according to maximum permissible limits stipulated by EOS(2005).

Table 1 Statistical analytical results of mold and yeast count (cfu/g) in the examined chilled and frozen duck meat samples (n = 100).

Duck meat samples		Min.	Max.	Mean ± S.E.*
Chilled	Breast	5.0×10	7.3×10^2	$3.1\times10^2 \pm 0.2\times10^{2b}$
	Thigh	8.0×10	1.1×10^3	$5.5\times10^2\pm 0.4\times10^{2a}$
Frozen	Breast	1.0×10	1.0×10^3	$3.0\times10^2\pm 0.3\times10^{2b}$
	Thigh	7.0×10	1.1×10^3	$6.0\times10^2\pm 0.4\times10^{2a}$

*S. E.= Standard Error of Mean. [ab] values within a column with different superscript letters were significantly different at ($P \leq 0.05$).

Table 2 Acceptability of the examined duck meat samples based on their mold and yeast count /g (n =50).

Duck meat sample	*PL	+ve		Accepted sample	
		No	%	No	%
Chilled breast		10	20	40	80
Chilled thigh		14	28	36	72
Frozen breast		32	64	18	36
Frozen thigh		38	76	12	24

*PL: Permissible limit according to Egyptian Organization for Standardization "EOS" (1651/2005) for chilled duck meat and EOS (1090/ 2005) for frozen duck meat.

Result in table (3) showed that incidence of mold genera isolated from examined duck meat samples of chilled and frozen samples (in breast and thigh) were *Aspergillus ochraceous* 2%, 4%, 8%, 0% and, *Aspergillus niger* 4%, 6%, 12% 16% and *Aspergillus fumigatus* 4%, 2%, 0% 10% and *Aspergillus flavus* 4%, 6%, 6% ,2% and *penicillium camberti*0%, 4%, 8%, 6% and *penicilliumpaxilli*4%, 0%, 6%, 2% and *Mucor spp.* 2%, 0%, 10%, 6% and*Alternaria*0%, 4%, 8%,14% and *Rhizopusspp.* 0%, 2%,6%, 10% .
It is evident from the results recorded in table (4) that incidence of yeast genera isolated from examined duck meat samples of chilled and frozen samples (in breast and thigh) ,respectively were *Saccharomyces spp*6%, 14%, 0%, 4% and *Candida tropicalis*2%, 12%, 4%, 2% and *Candida albicans*5%, 16%, 6% 4% and *Rhodotorularubra*2%, 8%, 2% 2% and *Rhodotorula Minuta* 10%, 14%, 0% 6% and *Rhodotorula mucilaginosa* 4%, 18%, 10%, 16%.

Table 3 Incidence of isolated mold genera from examined chilled and frozen breast and thigh duck meat samples (n=50).

Isolates	No. of positive samples				%			
	Chilled		Frozen		Chilled		Frozen	
	B	Th.	B	Th.	B	Th.	B	Th.
A. ocharceus	1	2	4	-	2%	4%	8%	-
A. niger	2	3	6	8	4%	6%	12%	16%
A. fumigatus	2	1	-	5	4%	2%	-	10%
A. flavus	2	3	3	1	4%	6%	6%	2%
Penicillium camberti	-	2	4	3	-	4%	8%	6%
Penicillium paxilli	2	-	3	6	4%	-	6%	12%
Mucor spp.	1	-	5	3	2%	-	10%	6%
Altrnaria	-	2	4	7	-	4%	8%	14%
Rhizopus spp.	-	1	3	5	-	2%	6%	10%
Total	10	14	32	38	20%	28%	64%	76%

B: Breast. Th.: Thigh. A.: Aspergillus.

Table 4 Incidence of isolated yeast genera from examined chilled and frozen duck meat samples (n=50).

Isolates	No. of positive samples				%			
	Chilled		Frozen		Chilled		Frozen	
	B	Th	B	Th	B	Th	B	Th
Saccharomyces spp.	3	7	-	2	6%	14%	-	4%
Candida tropicalis	1	6	2	1	2%	12%	4%	2%
Candida albicans	6	8	3	2	5%	16%	6%	4%
R. rubra	3	4	1	1	2%	8%	2%	2%
R. minuta	5	7	-	3	10%	14%	-	6%
R. mucilginosa	2	9	5	8	4%	18%	10%	16%
Total	22	41	11	17	44	82	22	34

B: Breast. Th.: Thigh. R: Rhodotorula

Fig. 1showed that there were three toxigenic strains out of 4 chosen samples**.** On the other hand, results in table (5) revealed that incidence of toxigenic strains of *Aspergillus spp.* isolated from examined chilled and frozen duck meat samples were *Aspergillusflavus*2%, 0%, 0%, 0% and *Aspergillus fumigatus* 0%, 0%, 0%, 2% and *Aspergillus niger* 0%, 0%, 0%,2% in breast and thigh ones.
Result in table 6showed,aflatoxin residues in examined duck samples were 0.26 ng /g in chilled and 0.33 ng /g in frozen breast but were not detected in frozen thigh samples.

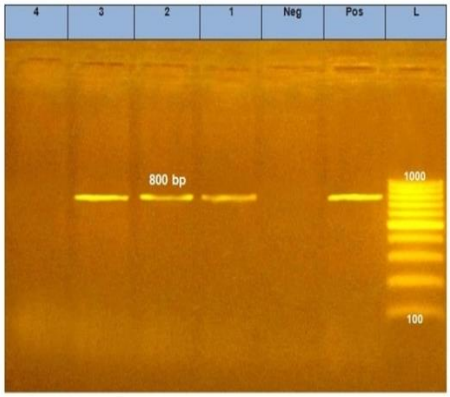

Fig. 1 Result of PCR amplifications. Lane L: 100-1000 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control (at 800 bp). Lan 1-3: Toxigenic strain positive samples

Table 5 Incidence of toxigenic strains of *Aspergillus spp.* isolated from examined chilled and frozen duck meat samples (n =50).

Isolates	Positive samples							
	===== Chilled =====				===== Frozen =====			
	Breast		Thigh		Breast		Thigh	
	No	%	No	%	No	%	No	%
A. flavus	1	2%	-	-	-	-	-	-
A. fumigatus	-	-	-	-	-	-	1	2%
A. niger	-	-	-	-	-	-	1	2%

Table 6 Aflatoxins residue in the examined duck meat samples (n = 50).

Duck meat samples		No. of positive samples	Toxigenic isolated strains	*PL	Amount of aflatoxin (ng/g)
Chilled	Breast	1	*A. flavus*	free	0.26
	thigh	-	-		-
Frozen	Breast	1	*A. niger*	free	0.33
	Thigh	1	*A. fumigatus*		0

*PL = permissible limit according Egyptian Organization for Standardization "EOS" (1651/2005) for chilled duck meat and EOS (1090/ 2005) for frozen duck meat

4. DISCUSSION

Molds are widely distributed in nature, both in the soil and in the dust carried by air. They may contaminate poultry meat at any stage of the production process and may render the product of inferior quality or even unfit for consumption, thus resulting in economic losses.

The result presented in table (1)revealed that yeast and mold count were higher in thigh samples than breast. Also, in chilled and frozen. This may be attributed to higher contamination from the ground and fecal matter. High counts may be due to unsanitary condition during preparation procedures, prolonged frozen storage period, contamination before and/or after slaughtering, bad handling during retail display and bad storage conditions or exposure to condition favoring mold proliferation and contamination of meat from different sources as skin of animals, pollution in abattoir atmosphere, visceral content in normal condition, transport and storage, halving, quartering, packaging utensil and also the water used for cleaning and personal uses (Thatcher and Clark, 1978).

According to our results, nearly similar results were obtained by Odetunde et al. (2011)(1.3×10^1 to1.5×10^2cfu/g inchilled chicken meat),Ogu et al. (2017)(1.3-4.0× 10^2cfu/g in frozen chicken meat) and Nossair et al. (2015) (1×10 to 9.1×10^3 with a mean value $3\times10^2\pm1.2\times10$ in frozen chicken).While higher results recorded by Hassan (2014) ($7.57\times10^2\pm1.06\times10^2$ and $1.12\times10^3\pm0.28\times10^3$cfu/g in duck breast and thigh), Omorodion Nnenna (2016) (2.7×10^4-5.9×10^5cfu/g in frozen chicken meat).But, lower results obtained by Capita et al. (2001)(2.99log10 cfu/g refrigerated chicken carcasses).These results disagree with Almorshidy (2013), who found that raw poultry samples were free of fungi and Darwish et al. (2016)(breast had the highest mold count than thigh).

Moreover, results in table (2) revealed that, the acceptable samples of frozen samples were lower than that of chilled ones. This may be due to intermittent freezing, temperature fluctuations in a storage works or improper ventilation which are common predisposing causes to mold growth.

Results achieved in table (3) illustrated that *Aspergillus*were the highest mold species isolated in both chilled and frozen samples, followed by *Penicillium, Mucor*, *Alternaria*, *Rhizopous spp*. They revealed that *Aspergillus spp.* isolated from examined duck meat were *A. niger* which was identified in high percentage in frozen samples. While, *A. flavus, A. fumigatus and A. ocharceus* were nearly equally isolated in fresh and frozen samples. This declared that freezing did not destroy Aspergillus. These results agreed with El-kewaiey (2014)(*A. flavus* and *A. niger*), Pencillum, Fusarium, Mucor were the most common fungi isolated from frozen duck meat, Rahal (2013), who said that highest recorded were Aspergillus 44(37%), followed by Penicilliumin chicken meat.Darwish et al. (2016) (The prevalent mold genera were *Aspergillus, Penicillium, Cladosporium*and *alternaria. Aspergillus niger, flavus, parasiticus* and *versicolor*were the identified Aspergilli In frozen chicken). But they werein a difference with those obtained by Abdel-Rahman et al.(1985) (*Penicillium, Caldosporium, Aspergillus,Mucor, Geotrichum, Thamnidium, Rhizopus, Paecilomyces, Scopulariopsis* and *Botrytis*were isolated from frozen poultry meat).

Moreover, results in table (4) illustrated that *Rhodotorula* was the highest yeast species in both chilled and frozen samples, followed by *Candida and Saccharomyces spp*. Isolated *Rhodotorula*spp.Were*Rhodotorulamucilginosa* which were identified in high percentage in frozen samples and *Rhodotorulaminuta* and *Rhodotorularubra* were higher in chilled samples. This may be due the different effect of cooling temperature in yeast genera .The previous result agreed with Hussein (1995) (the isolated yeast species were *Candida, Torulopsis, Rhodotorula, Saccharomyces* and *Trichosporon pullulans* with varying total percentagesfrom frozen poultry)and Shawish (2011) who isolated *candida* spp., *rhodotorula* spp., *saccharomyces* spp., *torulopsis* spp. from chicken cuts.But different results obtained by (Abdel-Rahman and Yassien, 1995)(isolated yeast genera were *Debaryomyces, Saccharomyces, Rhodotorula, Torulopsis, Endomyces, Trichosporon, Cryptococcus, Candida and Pichia*, respectively in frozen meat) and Rahal (2013), who isolated Candida as the highest total incidence followed by *Rhodotorula* and the lowest total incidence was *Saccharomyces*

Fig (1) showed that aflatoxin was found in 3 out of 4 (75%) samples. This result disagreed with Iqbal et al. (2014), who found that 35% of examined chicken samples contain aflatoxin, and El-kewaiey (2014)(10% of duck samples were positive to aflatoxins).

As shown in results in table (5) the incidence of toxigenic strains of *Aspergillus spp.* in frozen samples were high than in chilled ones. This declared that *Aspergillus* strains were not affected by low temperature and can grow well.

Result in table (6) showed that aflatoxin residues were higher in frozen duck samples than chilled ones. Moreover, breasts were higher than thigh samples which were free. This result was lower than Mohamed (2004)(8.10+0.71 ug/kg)(Pitt, 1984) reported that the total viable counts of molds are not a reliable indicator of mycotoxin production.

5. CONCULOSION

Finally, the present study concluded that duck carcasses can contribute to mycological risk and contamination. Consequently, frozen samples were more contaminated with fungi, the toxigenic strains and also aflatoxin residues were higher in frozen samples than chilled ones. Strict maintenance of good practices of hygiene, strengthened by

maintaining the cold chain is of central importance to ensure both public health protection and meat quality of ducks.

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