

PREVALENCE OF MYCOLOGICAL CONTAMINATION OF FRESH AND CHILLED RABBIT'S MEAT AND LIVER WITH SPECIAL REFERENCE TO MYCOTOXIGENIC STRAINS

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Abstract

At present, there is an increasing interest in public health due to the consumption of healthy and nutritious food that contains high protein, low cholesterol and fat. A total of 75 samples (50 carcasses of fresh rabbits with their livers and 25 carcasses of chilled rabbits) were purchased from various poultry shops and supermarkets in Giza governorate. Samples were tested for fungal contamination, detection of mycotoxin-producing strains, aflatoxin residues and comparing the obtained results by using both thin layer chromatography (TLC) and enzyme linked immunosorbent assay (ELISA). Seven genera of moulds and three genera of yeasts were isolated from the examined samples. The most isolated mould species were *Aspergillus* followed by *Penicillium*, *Scopulariopsis*, *Mucor*, *Rhizopus*, *Cladosporium* and *Geotrichum*. On the other hand, the most isolated yeast species were *Candida* followed by *Rhodotorula* and *Saccharomyces*. One isolate of the tested *Aspergillus flavus* obtained from both fresh rabbit carcass and its liver was an aflatoxin-producing strain. As well as extracted chilled rabbit meats did not contain any aflatoxin residues. The economic importance of the current findings and the importance of public health, as well as the application of proposed precautions to mitigate risks and reduce microbial contamination and ensure food safety for human health were discussed.

Key Words: Moulds, yeasts, rabbit's meat, rabbit's liver, chilled rabbit's meat, mycotoxigenic strains, aflatoxin residues, TLC, ELISA.

INTRODUCTION

The health of many people has been affected negatively after ingestion of contaminated food. To supply healthy meat without biological contamination to consumers is one of the most important aims in the food industry (Nakamura *et al.*, 2002). Worldwide production of rabbit meat is estimated to be over 1 million tons, marketed and consumed worldwide (Rodriguez - Calleja *et al.*, 2004). Rabbits seem as an important source for high protein which contains all essential amino acids required for human nutrition and water ratio with a low fat of unsaturated fatty acids and less cholesterol than other kinds of animal meat (Gergis, 2004).

Nistor *et al.* (2013) concluded that rabbit meat is healthier food over other meats frequently used in human nutrition and valued for its nutritional properties because of its high rich in proteins of high biological value, rich in calcium and phosphorus, low in fat, low in cholesterol content and high in linolenic acid, easy to digest in feeding children and old people (Dalle Zotte, 2000). Rabbit's meat is a source of B vitamins (B₂, B₃, B₅ and B₁₂) as reported by Combes (2004). A rare case of meat spoilage was identified in rabbit slaughterhouses.

Fungi are widely distributed in nature where they are introduced into animal tissues at the time of slaughtering and throughout meat processing for preparation of meat products (Davis *et al.*, 1980 and Mansour *et al.*, 1990) indicated that the growth of bacteria and fungi on a rich nutrient source as meat as well as the majority of meat spoilage by mould strains survived (lived after) freezing storage of meat and produced their special effect of mycotoxins by toxigenic fungi are favored only under certain environmental conditions at the favorable temperature, relative humidity or water activity, carbon dioxide, pH and oxidation reduction potential (Bullerman *et al.*, 1984 and Frazier and Westhoff, 1988).

Mycotoxins contaminated meats, either by direct fungal growth on meat consumed by human or by indirect carry over from animal feed to edible tissues (Pestka, 1986).

Hesham (2004) recorded that all raw rabbit meat samples were found to be contaminated with relatively high initial counts of aerobic mesophilic bacteria, psychrophilic bacteria, Enterobacteriaceae and moulds and yeasts as their mean log₁₀ cfu/g 10.00; 6.02; 5.88; 4.78 and 4.88, respectively. Rabbit production has economic importance as a way to supplement producers' income and to provide alternative sources of high quality food (Gebremedhin, 2009). It has been stated that many foods, particularly of animal origin, are heavily contaminated with microorganisms of various kinds (WHO, 1986 and 1989).

Mycotoxins are secondary toxic metabolites produced by toxigenic moulds that are capable of causing disease and death in animals and humans. Mycotoxins may enter the food supply by direct contamination resulting from mould growth on food or by indirect contamination through the use of contaminated ingredients in processed food industry. Mycotoxins are highly toxic, carcinogenic and mutagenic, and constitute a potential public health hazard to human being (Bullerman, 1979 and Youssef, *et al.* 1986).

Therefore, this work was planned to examine rabbit samples for mycological contamination, detection of mycotoxin-producing strains and aflatoxin residues and discuss the economic importance and public health significance.

MATERIALS AND METHODS

1. Collection of samples:

A total of 75 samples (50 fresh rabbit carcasses with their livers and 25 carcasses of chilled rabbits) were randomly purchased from various poultry shops and supermarkets in Giza governorate, in clean, dry and sterile polyethylene plastic bags and aseptically transported separately under refrigerated condition in ice box rapidly to the laboratory. Samples were maintained frozen at -20°C until analysis for mycological examination.

2. Preparation of samples:

Ten grams of rabbit meat were removed aseptically from each carcass of fresh rabbit's meat; fresh rabbit's liver and chilled rabbit's meat, minced and aseptically homogenized separately in a sterile (Braun Type) homogenizer containing 90 ml of 0.1% sterile peptone water broth as diluent's and homogenized at 2500 rpm for 2.5 minutes to prepare the initial 1/10 dilution (10^{-1}). One ml from each original sample homogenate was transferred into a sterile test tube containing 9 ml of 0.1% sterile peptone water and mixed carefully to provide a dilution of 10^{-2} . Tenfold serial dilution up to 10^{-4} was prepared (APHA, 2003).

From each previously prepared dilution, 1 ml was inoculated separately into a sterile Petri dish and mixed with sterile Dichloran-Rose Bengal Chloramphenicol agar (DRBC) or sterile Sabouraud's dextrose agar (SDA) containing 0.05 mg of chloramphenicol/ ml and left to solidify at room temperature after mixing, then incubate plates aerobically, in the incubator at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 days. Read the plates between 2 d and 5 d of incubation. The number of colonies of yeasts and moulds per gram or per milliliter is counted.

3. Enumeration of fungi (yeasts and moulds) – Colony count technique (cfu/g/ml):

Yeasts (TYC) (cfu/g/ml) and Moulds (TMC) (cfu/g/ml) were done according to ISO 21527/1 (2008). After incubation, colonies that developed on the plate were counted. The plates with (30 – 300) colony recorded as [Total fungi colony count (cfu/g/ml)].

4. Isolation and Identification of fungi:

4. 1. Isolation and identification of moulds:

The mould isolates were sub-cultured onto Malt extract agar (MEA) and Czapek-Dox agar then incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 days. The isolated mould colonies were selected, purified, identified individually by macroscopic (based on colony and cell morphology such as pigmentation, growth rate, texture shape and coloration on the

dorsal side reverse of the colony) and microscopic characteristics under oil immersion for septated or nonseptated hyphae, structure of hyphae, conidia, strigmata, vesicle head and conidiophores formation. The isolated mould genera and species were identified according to Samson *et al.* (1981) and Pitt and Hocking (2009).

4. 2. Isolation and identification of yeasts:

The isolated yeast colonies with yeast-specific morphology were identified using tests for growth sub-cultured on rice agar or corn meal agar and SDA, incubated at 37 °C for 2-3 days, production of a true mycelium, production of pseudo-mycelium, vegetative reproduction by budding or splitting, production of ascospores, production of basidiospores, fermentation and assimilation of sugars, nitrates assimilation and Urease hydrolysis. The isolated yeast genera and species were identified according to Kreger-Van Rij (1984) and Deak and Beuchate (1996).

5. Screening of aflatoxin producing *Aspergillus flavus* strains according to Hara *et al.* (1994).

5. 1. Qualitative and quantitative estimation of mycotoxins:

Production by cultivation and extraction of aflatoxins using 50 ml of sterile synthetic liquid medium (2% Yeast Extract, 20% Sucrose) (YES) and incubated at 25°C for 15-20 days and filtered, according to Pestka (1986).

6. Application of Thin Layer Chromatography (TLC) for determination of aflatoxins residues according to Schuller and Egmond (1991):

Fifty grams of rabbit carcasses examined samples were minced separately and extracted with 200 ml of methanol-water mixture (55:45) and filtered. Then 50 ml of the extract was placed in a separator funnel using 5ml of 1% NaCl, then extracted with duplicate by 50 ml of chloroform, shaking for 10 m_s and the aqueous lower layer evaporated to dryness in a water bath. Aflatoxins were then recovered with chloroform and spotted onto silica gel TLC plates before development with chloroform - acetone mixture (9:1) and the collected elute evaporated to dryness on a steam bath. Aflatoxin B₁, B₂, G₁ and G₂ reference standards were spotted across the plates. Fluorescence under long-wave length ultra violet light at (256 nm & 365 nm) (FUNA UV Light SL-800G) was used to examine the TLC plates so as to establish the presence of aflatoxins residues.

7. Procedure of ELISA tests (confirmation):

7. 1. Determination of Aflatoxins:

ELISA test kit is a competitive Enzyme Linked Immunosorbent Assay for the quantitative analysis of AFT_s in examined feed samples [RIDASCREEN® AFT Total (Art No.: R4701)] procedure as described by R- Biopharm Gmb H, and Darmstadt, Germany (Anonymous, 1999).

7. 2. Preparation of samples:

The samples should be stored in a cool place, protected against light. Fifty grams of each sample were triturated and mixed in a mixer separately following the test procedure and used directly in the test (antigen-antibody reaction). Two grams of grounded and homogenized composite samples were weighed and extracted with 10 ml methanol / distilled water (70/30; v/v) (70 %); mix and blend for 2 min in a high speed blender; filter the extract by a Whatman No. 1 filter paper; dilute 100 µl of the filtrate with 600 µl distilled water, put 50 µl per well. The following steps were done as RIDASCREEN instructions.

7. 3. Evaluation of Aflatoxins:

The absorbance values obtained for the standards and the feed samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (percentage maximum absorbance). The zero standards are equal to 100%, and the absorbance values were recorded in percentages. The values calculated for the standards were entered in a system of coordinates on semilogarithmic graph paper against the AFT concentration in ppb (ng/kg), the AFT concentration read from the calibration curve.

7. 4. Statistical Analysis:

Data were analyzed and results recorded as mean ± S.E. The calibration curve and line equation were prepared using available software, percentage, minimum, maximum and mean ± S.E were reported (Zar, 1984).

RESULTS AND DISCUSSION

Table 1. Statistical analytical results of Total Mould Counts in positive examined samples of rabbit carcasses and liver. (n. = 25 of each)

Type of examined samples.	Total Mould Count (cfu/g).				
	Positive samples		Min.	Max.	Mean ± S.E.
	No.	%			
Fresh rabbit's liver.	3	12	1.00×10^2	2.00×10^2	$1.60 \pm 0.60 \times 10^2$
Fresh rabbit's meat.	4	16	1.00×10^2	4.00×10^2	$2.17 \pm 1.27 \times 10^2$
Chilled rabbit's meat.	3	12	1.00×10^2	2.00×10^2	$1.50 \pm 0.50 \times 10^2$

cfu= colony forming unit/g/ml.

The results in table (1) showed that the mycological examination of (25 of each of) fresh rabbit's liver, fresh rabbit's meat and chilled rabbit's meat examined samples revealed that the highest incidence of moulds contamination was (16 %) in fresh rabbit's meat, followed by (12 %) in each of fresh rabbit's liver and chilled rabbit's meat. The highest mean value of total mould counts/ g was ($2.17 \pm 1.27 \times 10^2$ cfu/g)

in fresh rabbit's meat, followed by $(1.60 \pm 0.60 \times 10^2 \text{ cfu/g})$ in fresh rabbit's liver and $(1.50 \pm 0.50 \times 10^2)$ in chilled rabbit's meat.

These results agreed with Abo-Hussein (2014) reported that, mould count of the examined samples of fresh fore and hind quarters of rabbits ranged from 1.0×10^2 to 1.5×10^2 and 1.0×10^2 to 2.6×10^3 with mean values of $5.80 \times 10^2 \pm 1.06 \times 10^2 \text{ cfu/cm}^2$ and $6.50 \times 10^2 \pm 1.29 \times 10^2 \text{ cfu/cm}^2$, respectively. Also, the frozen fore and hind quarters ranged from 1.0×10^2 to 2.0×10^2 and 1.20×10^2 to 4.0×10^2 with mean values of $1.20 \times 10^2 \pm 0.17 \times 10^2 \text{ cfu/cm}^2$ and $1.60 \times 10^2 \pm 0.48 \times 10^2 \text{ cfu/cm}^2$, respectively. On the other hand, Tamer (2008) who stated that, the total mould counts in fresh and frozen rabbit samples by swab method ranged from 10 to 70 and 10 to 60 with a mean value of $35.8 \pm 2.6 \text{ cfu/cm}^2$, respectively.

Table 2. Incidence of mould species isolated from positive examined samples of rabbit carcasses and liver.

Isolated Mould species.	Type of examined samples.					
	Fresh rabbit's liver.		Fresh rabbit's meat.		Chilled rabbit's meat.	
	+ve. No.=3	%	+ve. No.=4	%	+ve. No.=3	%
<i>Aspergillus</i> spp.:	3	100	4	100	2	66.66
- <i>A. flavus</i> .	2	66.66	3	75	1	33.33
- <i>A. niger</i> .	1	33.33	1	25	1	33.33
<i>Cladosporium</i> spp	ND	0.0	ND	0.0	1	33.33
<i>Geotricum</i> spp.	ND	0.0	ND	0.0	1	33.33
<i>Mucor</i> spp.	ND	0.0	1	25	ND	0.0
<i>Penicillium</i> spp.	1	33.33	1	25	1	33.33
<i>Rhizopus</i> spp.	ND	0.0	1	25	ND	0.0
<i>Scopulariopsis</i> spp	ND	0.0	1	25	ND	0.0

%= Percentage was calculated in relation to the number of positive examined samples. ND= not detected.

Table (2) showed that the most isolated mould genera were *Aspergillus* (100%) from each of fresh rabbit's liver and fresh rabbit's meat and (66.66%) from chilled rabbit's meat, followed by *Penicillium* (33.33%) from each rabbit's liver and chilled rabbit's meat and then *Cladosporium* and *Geotricum*, (33.33%) from chilled rabbit,s meat only.

The members of genus *Aspergillus* were identified as *A. flavus* (75%) from fresh rabbit meat, (66.66%) from fresh rabbit's liver and (33.33%) from chilled rabbit's meat, followed by *A. niger* (33.33%) from each of fresh rabbit's liver and chilled rabbit's meat, and (25%) from fresh rabbit's meat.

These results agreed with Hesham (2004) who isolated moulds from rabbit carcasses out of which *Aspergillus* spp., *Mucor* spp. and *Penicillium* were the most common species isolated from samples and were often toxic. The low number of fungi in examined samples may be due to freezing of carcasses. Abo-Hussein (2014) who

reported that, the isolated mould species from fresh and frozen rabbit carcasses were *Aspergillus*, *Mucor*, *Penicillium*, *Alternaria*, *Fusarium*, *Geotrichum* and *Scopuloriopsis* spp. *Aspergillus* strains were identified as *A.flavus*, *A.fumigatus* and *A.niger*.

Washington (1990) stated that *Geotrichum candidum* could induce clinical symptoms such as conjunctivitis and bronchitis in humans. Moreover, Jay (2000) described *Rhizopus* species as spoilage microorganism of stored food that grows under anaerobic conditions causing black spots on frozen meat. It was frequently found as white to gray black cottony growth. Annadurai *et al.* (2003) reported that fungal contamination of meat with *Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Rhizopus* spp. and *Candida albicans* leads to the possible risk for Aspergillosis diarrhea and food poisoning. Hassan (2003) stated that growth of fungi in food cause a health hazard as they may produce mycotoxin, causing failure of liver and kidney functions and induction of cancer.

Table 3. Statistical analytical results of Total Yeast Counts in positive examined samples of rabbit carcasses and liver. (n. = 25 of each)

Type of examined samples.	Total Yeast Count (cfu/g).				
	Positive samples		Min.	Max.	Mean \pm S.E.
	No.	%			
Fresh rabbit's liver.	4	16	1.00×10^2	1.75×10^3	$9.55 \pm 1.33 \times 10^2$
Fresh rabbit's meat.	5	20	1.00×10^2	2.00×10^3	$1.03 \pm 0.77 \times 10^3$
Chilled rabbit's meat.	3	12	1.00×10^2	1.50×10^3	$7.41 \pm 3.22 \times 10^2$

cfu= colony forming unit/g/ml.

Table (3) showed that the highest incidence of yeasts contamination was 20 % in fresh rabbit's meat followed by 16 % in fresh rabbit's liver and 12 % in chilled rabbit's meat. The highest mean values of total yeast counts / g were $1.03 \pm 0.77 \times 10^3$ in fresh rabbit's meat, followed by $9.55 \pm 1.33 \times 10^2$ in fresh rabbit's liver and $7.41 \pm 3.22 \times 10^2$ in chilled rabbit's meat.

Similar results were reported by Jose *et al.* (2005) who found that the initial level of yeasts on chilled stored rabbit carcasses was $3.46 + 0.32 \text{ Log}_{10} \text{ cfu/g}$ which grew faster than the remaining microorganisms and became predominant at the end of the shelf life. Tamer (2008) stated that, the total yeast counts in fresh and frozen samples by swab method ranged from 20 to 50 and 30 to 70 with a mean value of 37.5 ± 5.44 and $50 \pm 14.14 \text{ cfu/cm}^2$, respectively. Abo-Hussein (2014) who reported that, the mean values of the total yeast count/ cm^2 in the examined swab samples of fresh and frozen fore and hind quarters of rabbits were ranged from $3.57 \times 10^3 \pm 0.84 \times 10^3 \text{ cfu/cm}^2$; $1.81 \times 10^3 \pm 0.25 \times 10^3 \text{ cfu/cm}^2$; $4.33 \times 10^2 \pm 0.62 \times 10^2 \text{ cfu/cm}^2$ and $3.86 \times 10^2 \pm 0.58 \times 10^2 \text{ cfu/cm}^2$, respectively.

Table 4. Incidence of yeast species isolated from positive examined samples of rabbit carcasses and liver.

Isolated yeast species.	Type of examined samples.					
	Fresh rabbit's liver.		Fresh rabbit's meat.		Chilled rabbit's meat.	
	+ve. No.=4	%	+ve. No.=5	%	+ve. No.=3	%
<i>Candida</i> spp.	2	50	4	80	2	66.66
<i>Rhodotorula</i> spp.	1	25	3	60	1	33.33
<i>Saccharomyces</i> sp	ND	0.0	2	40	ND	0.0

%= Percentage was calculated in relation to the number of positive examined samples. ND= not detected.

Table (4) showed that the most isolated yeast genera were *Candida* (80 %) from fresh rabbit's meat, (66.66 %) from chilled rabbit's meat and (50%) from fresh rabbit's liver followed by *Rhodotorula* (60 %) from fresh rabbit's meat, (33.33 %) from chilled rabbit's meat and (25%) from fresh rabbit's liver and *Saccharomyces* (40 %) only from fresh rabbit's meat.

These results agreed with Abo-Hussein (2014) who reported that, the isolated yeast species from fresh and frozen rabbit carcasses were *Candida*, *Rhodotorula*, *Sacchromyces* and *Torulopsis*.

Flee (1990) concluded that some species of yeast especially some members of *Candida* constitute a public health hazard as they may be incriminated in case of pulmonary infection, urinary tract infection, endocarditis, eye infection, nail affection, thrush in mouth, gastrointestinal disturbance, vulvo-vaginitis, arthritis, osteomyelitis, dermatitis, meningitis and occasionally fatal systemic disease. Dillon and Board (1991) mentioned that, some species of *Saccharomyces* and *Rhodotorula* might cause fungemia, fatal endocarditis and Mycotic keratitis.

James (2000) added that, *Candida* is normal flora of the digestive and urogenital tract of human. *Candida albicans* cause vaginal yeast infection, thrush in the mouth, also invades the lungs, kidneys, heart or carried in the blood where it caused severe toxic reaction. Candidiasis is the most common nosocomial fungal infection, and seemed in patients who have tuberculosis, leukemia and aids.

Table 5. Measurement of toxigenicity of isolated *A. flavus* from positive examined samples of rabbit carcasses using Thin Layer Chromatography (TLC).

<i>A. flavus</i> .	Type of examined samples.		
	Fresh rabbit's liver.	Fresh rabbit's meat.	Chilled rabbit's meat.
	+ve. No.= 2	+ve. No.= 3	+ve. No.= 1
Toxigenic.	1	1	ND
Non- Toxigenic.	1	2	1

Tables (5) revealed that only one isolate of *A. flavus* obtained from both of fresh rabbit liver and fresh rabbit meat were aflatoxin producers using TLC, this indicates clearly that not all *A. flavus* isolates could be able to produce aflatoxins, while *A. flavus* isolates obtained from chilled rabbit's meat can not produce aflatoxins.

These results were in agreement with those recorded by (Pestka, 1986) who stated that the presence of toxins producing moulds in meat does not necessarily mean that aflatoxins are present. Farghaly (1998) isolated 482 mould strains from 100 random swabs samples obtained from different meat cold stores. The isolated *A. flavus* group was screened for the toxin production where 47.7 % of them were found to be aflatoxin producer. In this respect, Abo-Hussein (2014) who showed that toxigenicity of *A. flavus* isolated from examined surfaces of rabbit carcasses samples of fresh and frozen samples (in fore and hind quarter), respectively were toxigenic 24 %, 0 %, 0 % & 8 % and non-toxigenic 16 %, 4 %, 4 % & 0 %, where the mean values of aflatoxins ($\mu\text{g/L}$) extracted from the toxigenic strains of *A. flavus* were 41.04 ± 1.65 ($\mu\text{g/L}$) for B₁; 29.87 ± 1.26 ($\mu\text{g/L}$) for B₂; 15.41 ± 0.90 ($\mu\text{g/L}$) for G₁ & G₂ 9.38 ± 0.49 ($\mu\text{g/L}$) in fresh fore quarters of rabbits, and B₁ 76.85 ± 3.28 ($\mu\text{g/L}$); B₂ 57.14 ± 2.39 ($\mu\text{g/L}$); G₁ 40.53 ± 2.12 ($\mu\text{g/L}$) & G₂ 19.66 ± 1.25 ($\mu\text{g/L}$) in frozen hind quarters of rabbits, respectively. Moreover, Giradin (1997) reported that fungi are not only implicated in meat spoilage, but also constituted a real risk to public health due to the production of mycotoxins. Pitt and Hocking (2009) stated that aflatoxins regarded as a potent toxin has a carcinogenic, teratogenic and mutagenic effects to humans.

Table 6. Comparison between mean levels of Aflatoxin (AFT) residues (ppb= $\mu\text{g/kg}$) extracted from positive examined samples using TLC and ELISA. (n. = 5 of each).

AFT	Type of examined samples.					
	Fresh rabbit's liver.		Fresh rabbit's meat.		Chilled rabbit's meat.	
	<i>A. flavus</i> +ve.= 2	%	<i>A. flavus</i> +ve.= 3	%	<i>A. flavus</i> +ve.= 1	%
	1	50	1	33.33	ND	0.0
	Mean ± S.E.		Mean ± S.E.		Mean ± S.E.	
	TLC	ELISA	TLC	ELISA	TLC	ELISA
AFB ₁	3.26 ± 1.06	4.50	2.04 ± 1.10	3.40	ND	ND
AFB ₂	2.94 ± 1.22	4.15	ND	ND	ND	ND
AFG ₁	2.84 ± 0.60	3.50	1.18 ± 0.34	1.55	ND	ND
AFG ₂	1.66 ± 0.12	2.30	ND	ND	ND	ND

Table (6) showed that the highest total aflatoxin residues were detected in one positive examined sample of fresh rabbit's liver (50 %), the highest amount of aflatoxin recorded 3.26 ± 1.06 ppb and 4.50 ppb by using TLC and ELISA respectively in case of AFB₁; 2.94 ± 1.22 ppb and 4.15 ppb by TLC and ELISA in AFB₂; 2.84 ± 0.60

ppb and 3.50 ppb by TLC and ELISA in AFG₁ and 1.66 ± 0.12 ppb and 2.30 ppb by TLC and ELISA in AFG₂. Also, the total aflatoxin residues extracted from 1 positive examined sample of fresh rabbit's meat (33.33 %), the mean level of AFT was 2.04 ± 1.10 ppb and 3.40 ppb by TLC and ELISA in AFB₁ and 1.18 ± 0.34 ppb and 1.55 ppb by TLC and ELISA in AFG₁, while AFB₂ and AFG₂ were not detected. As well as extracted chilled rabbit meat can not produce any aflatoxin residues.

Application of Thin Layer Chromatography (TLC) is a semi qualitative and quantitative estimation of aflatoxins, an inexpensive, quick, and reliable. ELISA (Enzyme linked immunosorbent assays) kit is an inexpensive, quick, reliable, used for qualitative and quantitative analysis of AFT_s and highly valuable tool to monitor and ensure food safety worldwide.

FAO (1995) and FDA (1999) recorded that valid limit for AFT_s acc. to EU equal 2 ppb for AFB₁ and 4 ppb for all AFT_s in total cereals and feed for human consumption. FAO (2010) recorded that African maximum permissible limits were 5µg/kg for AFB₁ and 20µg/kg for total AFT in human foods.

These results agreed with Mahmoud *et al.* (2001) who stated that aflatoxins were detected in 2 samples of livers of poultry with mean of 36 µg/ kg.

Aflatoxins are secondary carcinogenic metabolites of the mould species *Aspergillus (flavus, parasiticus and nomius)*. The presence of aflatoxin residues in meat due to ingestion of low levels of aflatoxin over extended periods constitutes a public health hazard (Refai, 1988). The consumption of the contaminated meat by moulds and their mycotoxins induces hemorrhages with hepatotoxic, teratotoxic, carcinogenic or hormonal effects and immunosuppressant (Cheo, 1991). Galvano *et al.* (2005) stated that the occurrence of mycotoxins in human foods (breast milk, milk and milk products, meat and meat products, eggs, cereals, wine, beer, fruits and fruit juices, coffee, cocoa products and spices) are associated risks in conditions such as nephropathy, hepatocellular carcinoma and esophageal cancer.

So, all the preparative steps of the meat, from the time of food animal slaughter until it is ready-to-cook, should be placed under ideal conditions of hygiene (Stiles and Ng, 1981). The processing, production, transportation and sale of meat products must be performed with good care to a hazard analysis critical control point (HACCP) to prevent any hazard or health risks to the consumer (Madden, 1994).

Egyptian Organization for Standardization and Quality (2015) stated that frozen poultry and rabbits should be stored at a temperature not exceeding -18 °C, relative humidity not less than 90%, the storage period is not more than 9 months. The surface of frozen poultry and rabbits should be completely free of viscous exudate or fungal or bacterial growth or any sign of spoilage, rancidity or any unacceptable odour.

CONCLUSION AND RECOMMENDATION

Rabbit meat is healthier, high in protein and low in fat. Safe production of rabbit meat is necessary from good clean healthy feeding during growth, rabbit meat market and production until it reaches the consumer. Strict hygienic measures and maintenance of good practices of slaughter hygiene, maintaining the cold chain during transport and distribution should be recommended to ensure both public health protection and good meat quality. Strict hygienic measures should be recommended to the display refrigerators in markets. The most effective means of eliminating human exposure to mycotoxins in foods is by the prevention and reduction of mycotoxins formation.

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مدي التلوث الفطري فى لحوم وأكباد الأرناب الطازجة والمبردة مع الإشارة الخاصة للعترات المفترزة للسموم

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فى الوقت الحاضر، هناك إهتمام متزايد بالصحة العامة بسبب إستهلاك الطعام الصحى والمغذى الذى يحتوى على نسبة عالية من البروتين، ومنخفض من الكوليسترول والدهون. لذا أجرى هذا البحث على عدد ٧٥ عينة (٥٠ ذبيحة من الأرناب الطازجة مع أكبادها و ٢٥ ذبيحة من الأرناب المبردة) من مختلف محلات الدواجن و السوبر ماركت فى محافظة الجيزة. تم إختبار العينات للتلوث الفطري، والكشف عن السلالات المنتجة للسموم الفطرية، وبقايا الأفلاتوكسين، ومقارنة النتائج التى تم الحصول عليها بإستخدام كل من تطبيق كروماتوغرافيا الطبقة الرقيقة وتقنية إختبار الإرتباط الانزيمى المناعى (الإليزا). تم عزل سبعة أجناس من الأعفان من العينات التى تم فحصها وثلاثة أجناس من الخمائر. كانت الأعفان الأكثر عزلا هي أسبرجيلس تلبها بينيسيليوم، سكوبولاريوبسيس، ميكور، ريزوبس، كلادوسبوريوم وجيوتريكوم. من ناحية أخرى، كانت أنواع الخمائر الأكثر عزلا هي الكانديدا تلبها رودتورولا وساكارومييسيس. عزلت عترة واحدة من الأسبرجيلس فلافس المختبرة التى تم الحصول عليها من كل من ذبيحة أرناب طازجة وكبده كان منتج للأفلاتوكسين. وجد أن لحوم الأرناب المبردة لا يمكن أن تنتج أي بقايا للأفلاتوكسين. نوقشت الأهمية الإقتصادية للنتائج الحالية وأهمية الصحة العامة لها، فضلا عن تطبيق الإحتياطات المقترحة لتخفيف المخاطر والحد من التلوث الميكروبي وضمان سلامة الأغذية لصحة الإنسان.