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HYGIENIC STATUS AND PREVELANCE OF HEAVY METALS AND PESTICIDE RESIDUES IN FROZEN MEAT, CHICKEN AND THEIR PRODUCTS IN LUXOR CITY

(With 7 Tables)

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الحالة الصحية ومدى تواجد بقايا المعادن الثقيلة والمبيدات الحشرية فى اللحوم المجمدة والفراخ المجمدة ومنتجاتها فى مدينة الاقصر

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تم تجميع عدد خمسة وسبعون عينة لحوم مجمدة ولحم مفروم بقرى مجمد وفراخ مجمدة (٢٥ عينة من كل نوع) من أسواق مدينة الاقصر خضعت للفحوص الكيميائية والبكتيريولوجية وكان تركيز الاس الهيدروجينى ونسبة القواعد النيتروجينية الكلية المتصاعدة فى الحدود المسموح بها. وكان متوسط قيم المعادن الثقيلة (الكادميوم والرصاص والزئبق) ٠٠٥, ، ٠١٢, ، ٠٠٣, ، ٠٢٢, ، ٠٠٤, ، ٠٣٥, ، ٠٠٩, ، ٠٠٦, ، ٠٨٥, ميكروجرام / جرام على التوالى وهى فى الحدود المسموح بها، وكان متوسط تركيز دى دى تى ، ألدرين +داى ألدرين ولينـدان ٢.١، ٤. ، ١.٣ ميكروجرام/كيلو جرام فى اللحوم البقرية و ٣, ، ١.٢ ، ١.٦ ميكروجرام /كيلو جرام فى اللحم المفروم البقرى وغير ملحوظة فى الفراخ وكان متوسط العد البكتيرى الكلى للميكروبات الهوائية والميكروبات المعوية والميكروبات القولونية (العد الاحتمالى) فى اللحوم البقرية المجمدة ، واللحم المفروم البقرى ، الفراخ ٢.٨×١٠^{٦} ، ١.٦×١٠^{٦} ، ٢×١٠^{٦} ; ٢.١×١٠^{٤} ، ٢.٧×١٠^{٤} ، ٢×١٠^{٤} ; ١.٥×١٠^{٣} ، ٣.١×١٠^{٣} ، ١.٢×١٠^{٣} خلية بكتيرية/ جرام من العينة على التوالى. وكانت جميع العينات خالية من ميكروب السالمونيلا. وكان تواجد الميكروب المكور العنقودى الذهبى والايشيريشيا كولاى المرضية فى عينات اللحوم البقرية المجمدة ، اللحم المفروم البقرى والدواجن المجمدة ٨ ، ٢١.٣ ، ٦.٧ ، ٤ ، ٨ ، ٤% على التوالى.

SUMMARY

Seventy five frozen beef, minced beef and chicken samples (25each) were collected from markets located in Luxor City, and subjected to chemical and bacteriological examinations. The PH and TVB-N were within the permissible limits.The examined samples had a normal values of Cd, Pb and Hg (the mean values were 0.005, 0.012, 0.003; 0.022, 0.004, 0.035 and 0.009, 0.006, 0.085 µg / g respectively). The mean concentration of DDT, Aldrin+dieldrin and Lindane were 2.1, 0.4 and 1.3µg / kg in beef and 3.0, 1.2 and 1.6µg / kg in minced beef and were undetected in chicken. The mean values of Aerobic plate, Enterobacteriaceae and Total coliform (MPN) counts of frozen beef, minced beef and chicken were $2.8x10^6$, $1.6x10^6$, $2x10^6$; $2.1x10^4$, $2.7x10^4$, $2x10^4$; $1.5x10^3$, $3.1x10^3$ and $1.2x10^3$ CFU/g respectively. All the samples were free from salmonella. The incidence of staph. aureus and Enteropathogenic E.coli in frozen beef, minced beef and chicken samples were 8 , 21.3 , 6.7 ; 4 , 8 and 4% respectively.

Key words: *Heavy metals, pesticide residues, frozen meat, chicken, meat products*

INTRODUCTION

Traditional physical meat inspection in markets is based on visual inspection, which does not identify pathogenic microorganisms such as Salmonella, Enteropathogenic E.coli, or Staph.aureus (Edwards, *et al.* (1997). To improve the control of such pathogens via improvement in process hygiene must apply the hazard analysis critical control point (HACCP) system which has been promoted and implicated in the European Union (EU)**,** (Anonymous, 1999). This analysis allowed the identification in the process flow of the sensitive areas that might contribute to a hazard. During processing of meat may become contaminated with both spoilage and pathogenic microorganisms from fecal and stomach contents. Additional sources of microbial contamination are the processing tool, equipments, structural components of the facility, human contact and carcass to carcass contact (Institute of food technologists, 2002).

The toxic elements cadmium, lead and mercury are widely distributed in environment and generally regarded as accidental pollutants although they are frequently found in minute amounts in food (Lucis *et al.,* 1972, Underwood, 1977). Cadmium is used extensively in the mining and electroplating industries and found in fertilizers and fungicides. All its chemical form are toxic (Fleischer *et al.,* 1974). It inhibits sulphydryl enzyme systems necessary for cellular metabolism (Gunn and Gould, 1957).

Lead is used in many industrial processes, lead paint, lead gasoline. It is a common material for spraying fruit trees. Absorbed lead accumulates in tissue of animal. Lead inhibits the activity of enzymes dependent upon the presence of free sulphydryl groups for their activity and this inhibition is clearly demonstrated in disturbance which occur in the biosynthesis of heme (Klauder and petering, 1975, Willoughby *et al.*, 1976).

Mercury was used as fungicides, mercurial fungicides used for seed dressing. Toxic compounds of mercury accumulate in animal tissues, the alkylmercuries are slowly metabolized and more evenly distributed in the body tissues (Underwood, 1977). Mercury caused redness of lips, throat and tongue, loss of teeth, swelling and redness of the skin with pink-red finger tips. it affects the nervous system causing irritability (Mert, 1987). Contamination of food of animal origin by organochlorine and organophosphorus compounds and their metabolites has been reported in various countries (Neumann, 1988 and Goldman *et al.,* 1990). The main side effect of environmental pollution by pesticides in food contamination leading to injury of non-target organisms concerns the health of the workers and consumers. The wide spread usage of pesticides in Egypt led to many problems and constituted hazard in animals. In recent years, pesticides in food arises as an important problem of serious public health hazards which may lead to acute or chronic hepatic toxicity for human being.

The trend in this study is to evaluate the hygienic status and detection of heavy metals and pesticide residues in frozen (beef, minced beef and chicken) in Luxor City markets.

MATERIALS and METHODS

A - Sampling

Seventy five frozen beef, frozen minced beef and frozen chicken samples (25 each) were collected from markets located in Luxor City. The samples were transferred to the laboratory in an ice box without undue delay to be examined.

B- Chemical examination:

- **a-** Determination of pH value according to ISO (1974)
- **b-** Determination of Total Volatile Basic Nitrogen (TVB-N) according to FAO (1992).
- **c-** Heavy metals analysis: Digestion of samples was according to Perez (1999), Determination of cadmium (Cd), lead (Pb) and mercury (Hg) were conducted by using atomic absorption

spectrophotometer "AAS" (Perkin Elmer, 2380, USA) which was adjusted at 228.8, 217 and 253.7 nm for Cd, Pb and Hg respectively.

d- Determination of Organochlorine (OC) and organophosphrous (OP) pesticides were conducted by using HPLC apparatus (ISCO model 2350) HPLC and 205 UV/vis detectors with Hypersil HPLC column 250 x 4.6 mmBDs 18 OC 5M.

Samples were extracted and the pesticides residues were determined according to A.O.A.C. (1980) and Pesticide Analytical Manual (PAM) (1994).

C- Bacteriological counts:

The samples were subjected to bacteriological examination through determination of:

a- Aerobic plate count by spreading technique according to European Union Communities Commission (2001).

b- Enterobacteriaceae count according to European Union Communities Commission (2001).

c- Enumeration of coliform bacteria by Most Probable number (MPN) according to FAO (1992).

D- Detection of some food-borne pathogens:

a- Isolation and identification of Staphylococcus aureus according to Bennett and Lancette (2001).

b- Isolation and identification of Salmonellae according to Health Protection Agency (HPA) (2003 a).

c- Isolation and identification of Enteropathogenic Escherichia coli according to Health Protection Agency (HPA) (2003 b).

Serological identification was done by using diagnostic sera, (Biotec, 1999).

RESULTS

Table 1: Mean values of the pH and TVB-N of the examined samples (n = 25 each)

Samples / Test	Frozen beef	Frozen minced beef	Frozen chicken
pH	5.9	6.1	5.8
TVB-N mg/100gm	13	16	20

Table 2: Concentration of heavy metals in the examined samples (μg /g). (n=25 each)

	Frozen beef			Frozen minced beef			Frozen chicken		
	Cd	Pb	Hg	Cd	Pb	Hg	Cd	Pb	Hg
Min	0	0.006	0.005	0	0.001	0.005	0	0.02	0.08
Max	0.04	0.045	0.099	0.035	0.007	0.008	0.02	0.05	0.1
Mean	0.005	0.022	0.009	0.012	0.004	0.006	0.003	0.035	0.085
SE	0.004	0.002	0.004	0.003	0.002	0.002	0.004	0.004	0.004

Table 3: Chlorinated pesticide residues in the examined samples in (ppb)

Samples		Organochlorine pesticides		
		DDT	Aldrin+dieldrin	Lindane
Frozen beef	Min	0.7	0.02	0.65
	Max	3.5	1.0	2.5
	Mean	2.1	0.4	1.3
	SE	0.04	0.04	0.04
Frozen minced beef	Min	0.9	0.8	1.1
	Max	4.5	2.0	2.5
	Mean	3.0	1.2	1.6
	SE	0.02	0.02	0.02
Frozen chicken		N.D*	N.D*	N.D*

* Not detected.

Table 4: Organophosphorus pesticide residues in the examined samples (ppb)

Samples		Organophosphrous pesticides	
		Malation	Dimethoate
Frozen beef	Min	1.0	0.02
	Max	2.5	1.00
	Mean	1.4	0.35
Frozen minced beef	Min	1.5	0.26
	Max	2.8	1.2
	Mean	1.7	0.7
Frozen chicken	Min	0.8	N.D*
	Max	1.2	
	Mean	0.95	

* Not detected

Table 5: Mean values of bacterial counts of the examined samples (n = 25 each)

Samples / Counts	Frozen beef	Frozen minced beef	Frozen chicken
Aerobic plate	$2.8x10^{6}$	$1.6x10^{6}$	$2x10^{6}$
Enterobacteriaceae	$2.1x10^{4}$	$2.7x10^{4}$	$2x10^{4}$
Coliform (MPN)	$1.5x10^{3}$	$3.1x10^{3}$	$1.2x10^{3}$

Table 6: Incidence of food-borne pathogens in the examined samples (n = 25 each)

Samples / Isolated M.O.	Frozen beef		Frozen minced beef		Frozen chicken	
	+ve	%	+ve	%	+ve	%
Staph-aureus	6	8	16	21.3	5	6.7
Salmonella	0	0	0	0	0	0
Enteropathogenic E.coli	3	4	6	8	3	4

Table 7: Serotyping of Enteropathogenic E.coli isolated from the examined samples

Sample / Serotypes	Frozen beef	Frozen minced beef	Frozen chicken
O_{55}	1	1	0
O_{128}	1	1	0
O_{111}	1	1	0
O_{114}	0	1	1
O_{119}	0	1	1
O_{142}	0	1	1

DISCUSSION

Table (1) showed that the mean values of the pH and TVB-N (mg/100g) of the examined frozen beef, frozen minced beef and frozen chicken were 5.9 , 13 ; 6.1 , 16 and 5.8 , 20 respectively. The obtained data are within the permissible limits according to The Egyptian Standards (1991, 2005) {pH is 5.6 -6.2 and TVB-N is 20 mg/100g}.

Data presented in Table (2) showed that the examined samples had a normal values of Cd, Pb and Hg when compared by FAO/WHO (1972) dietary intake limits (Cd 20 – 100 µg / day, Pb 100µg / day and Hg 0.03 mg / day). Egyptian Organization for Standardization and Quality control E.O.S.Q.C (2360/1993) mentioned that the maximum provisional weekly intake from cadmium by human as 0.0067 – 0.0083 mg/kg of body weight and 2mg/kg of sample weight, and from lead by human as 0.05 mg / kg body weight.

Cadmium (Cd) concentration in beef, minced beef and chicken in Table (2) ranged from 0.0 to 0.04, 0.0 to 0.35 and 0.0 to 0.02 µg / g with mean values of 0.005, 0.012 and 0.003 µg /g respectively. These results agreed with those reported by Folandyezand Lorenc-Biala (1991) and Salisbury *et al.* (1991) and lower than that reported by Kienholz *et al.* (1974), Doyle and Pfander (1975), Wright *et al.* (1976) and Daoud *et al.* (1998).

Lead (Pb) concentration ranged from 0.006 to 0.45, 0.001 to 0.007 and 0.2 to 0.05µg / g with means 0.022, 0.004 and 0.035 µg / g in beef, minced beef and chicken samples respectively. These results agreed with the results which reported by Spaulding (1975), Wright *et al.* (1976) and Salisbury *et al.* (1991) and lower than Amodio-

Coccheri and Fiore (1987), Folandyez and Lorenc-Biala (1991) and Schiilz-Schroeder (1991).

Shroeder and Hipton (1968) have reported the significant difference in the levels with age as Pb concentration increased with age in many tissue under USA environmental conditions as cited by Underwood (1977).

Mercury (Hg) conc. in beef, minced beef and chicken as recorded in Table (2) ranged from 0.005 to 0.099, 0.005 to 0.008 and 0.08 to 0.1 µg / g with mean values of 0.009, 0.006 and 0.085 respectively. The recorded results of Hg agreed with those reported by Sell *et al.* (1975), Spaulding (1975) and National Bureau of Standards (1976).

Cd, Pb and Hg concentrations in the examined samples were within the permissible limits.

In Table (3) the existence of the Organochlorine pestisides with varying concentrations, reflects the intake of pesticides by the animals and may be attributed to way of nutrition (graze in different pastures) and continuously exposure to the spraying with insecticides to control external parasites. The persistence of DDT in the environment means that much of the material used for control of insect borne diseases and elimination of agricultural pests still contaminates soil, water and air (Ralls and Cortes, 1972) though their wide spread use in this country was restricted in 1974. Food and Feeds still contain detectable levels of these contaminants. Results recorded in Table (3) show that the concentration of DDT, Aldrin, Dieldrin and Lindane in beef ranged from 0.7 to 3.5, 0.02 to 1.0 and 0.65 to 2.5 mg /kg respectively, while in minced beef were 0.9 to 4.5, 0.8 to 2.0 and 1.1 to 2.5 with mean values of 2.1, 0.4 and 1.3 mg / kg in beef and 3.0, 1.2 and 1.6mg / kg in minced beef. DDT, Aldrin, Dieldrine and Lindane were undetected in chicken, the concentration of DDT in samples analyzed were much lower than the maximum limits established by the World Health Organization (1989) (5ppm in meat) DDT is still ubiquitous in the environment due to its past wide use and its chemical and physical characteristics. DDT persists for more than 10 years in the soil and accumulates in the organisms through the food chain. After absorption, a part of the DDT is metabolized, the products in mammals being DDE, DDA and DDT (Bartik and Piskac, 1980). Low levels of DDT and its metabolites will be present in the environment for a long time (Ert and Sullivan, 1992). The concentration of Lindane was low in beef than minced beef while their mean values were higher than the maximum limits established by the (WHO, 1989) which is 1.1 ppm. The data agrees with that recorded by Zasadowski *et al.* (1991). The mean concentration of Aldrin, Dieldrine in the examined samples was higher than the maximum limits established by (WHO, 1989) which is 1.01 ppm. Similar results were nearly obtained by El-Shafei (1988) and Folandyez and Kannan (1992).

Table (4) shows the concentration of Malathion and Dimethoat which ranged from 1.0 to 2.5, 0.02 to 1.0 µg / kg in beef, respectively, while in minced beef ranged from 1.5 to 2.8 and 0.26 to 1.2 µg / kg, respectively, with mean values of 1.4 and 0.35 µg / kg in beef while mean of malation and dimethoate in minced beef was 1.7 and 0.7 µg / kg respectively, this result lower than Frank *et al.* (1990). Consequently, organochlorin and organophosphrous pesticide residues in food of animal origin are substantially high in developing countries than in areas of intensive urban due to application of pest control programs with care under official supervision.

The results presented in Table (5) showed that the mean values of aerobic plate, enterobacteriaceae and coliform (MPN) counts of frozen beef, frozen minced beef and frozen chicken were$2.8x10^6$, $1.6x10^6$, $2x10^6$; $2.1x10^4$, $2.7x10^4$, $2x10^4$; $1.5x10^3$, $3.1x10^3$ and $1.2x10^3$ CFU/g respectively. Bosilevac *et al.* (2004) reported that the mean values of aerobic plate and enterobacteriaceae counts in beef were $9.5x10^5$ and $6.1x10^4$ respectively. Arther *et al.* (2004) reported that the mean values of aerobic plate and enterobacteriaceae counts in beef were $2.9x10^3$ and 250 respectively. Gill *et al.* (2005) reported that the aerobic plate and coliform counts of chicken were 10^3 and 20 respectively. Pepperell, (2005) reported that the aerobic plate count of cattle was $0.8x10^4$. Paulsen *et al.* (2006) reported aerobic plate count of beef minced meat ranged from $0.2x10^2$ to $0.5x\ 10^7$ CFU/g.

Table (6) showed that the incidence of staph. aureus, salmonella and Enteropathogenic E.coli of the examined frozen beef, frozen minced beef and frozen chicken samples were 8 , 21.3 , 6.7 ; 0, 0, 0, ; 4 , 8 and 4% respectively. Ploatjies *et al.* (2004) identified staph.aureus in 50% of the beef carcasses. The high levels of staph.aureus growth indicate poor hygiene of the meat handlers during the processing stage as well as lack of sterilization of utensils and working surfaces. Staph.aureus produce enterotoxins which lead to food poisoning. Yasmine *et al.* (2005) isolated 4 salmonella strains out of 120 beef samples at a percentage of 3.3%. Busani *et al.* (2005) isolated 49 salmonella strains out of 5037 beef samples at a percentage of 1%. Phillips *et al.* (2001) recovered 10.3% Enteropathogenic E.coli out of 1275 beef samples. Table(7) showed that the isolated Enteropathogenic E.coli serotypes were two strains each of O_{55}, O_{128}, O_{111}, O_{114}, O_{119} and O_{142}. George (2004) reported that Enteropathogenic E.coli serotypes O_{55} and O_{111} were imbilicated as a causative agents for nursery school outbreaks in London and Aberdeen in the 1940's. He also added that O_{111}, O_{119}, O_{128} and O_{142} serotypes were isolated from infected infants in North America and Europe during the 1950's and up to the early 1970's in the United Kingdom. These outbreaks had a high attack rate and characterized by high mortality rate, which could exceed 50% of the babies less than 6 months of age, especially those that were bottle-fed.

In conclusion, frozen beef, minced beef and chicken samples showed high bacterial loads beside a relatively high rate of the pathogens, this is due to miss-handling and processing as well as the negligence of hygienic aspects at the production level. There fore, one can safely recommend the following, aiming to have meat with good quality: good hygiene of the meat handlers during the processing stage as well as good of sterilization of utensils and working surface. Thermo-stable of refrigerators and deep freezer is important for retarding the growth of both pathogenic and spoilage bacteria. Each food item must be kept separate. The laboratories performing the analysis must be accredited according to the ISO standard. Food handlers need to be educated on the importance of proper, safe hygienic working practices.

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