Heavy Metals in some Meat Products and Its Biocontrol by Probiotic Strain *Enterococcus Faecium* (M74) in Minced Meat

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A B S T R A C T

This study was conducted to detection of heavy metals and its biocontrol by probiotic strain *enterococcus faecium M74*. A grand total of 120 random samples of meat products represented by beef burger, beef sausage, beef minced meat and beef kofta (30 of each) were collected from different supermarkets located in Cairo and Giza governorate. The average of lead, cadmium and arsenic levels (ppm) were 0.13 ± 0.01, 0.07 ± 0.01 and 0.04 ± 0.01 in the examined beef minced meat, respectively, 0.24 ± 0.01, 0.11 ± 0.01 and 0.04 ± 0.01 in the examined beef kofta, respectively, 0.21 ± 0.01, 0.18 ± 0.01 and zero in the examined beef burger, respectively and 0.36 ± 0.02, 0.16 ± 0.02 and 0.10 ± 0.02 in the examined beef sausage, respectively. The *E. faecium* strain (M74) removed lead and cadmium efficiently from the minced meat. Lead content (50 ppm) in the group treated with *E. facieum* (M74) showed reduction after 8, 16 and 24 hrs of incubation, their reduction rate was 66.4%, 73% and 82.6%, respectively. While, cadmium content (10ppm) in the group treated with *E. facieum* (M74) showed reduction after 8, 16 and 24 hrs of incubation, their reduction rate was 59%, 67% and 71%, respectively.
Key words: meat, Nutritional criteria, preservatives, *H*eavy metals, *Enterococcus faecium M74*.

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1. INTRODUCTION

Improvements in the food production and processing technology had increased the chances of contamination of food with various environmental pollutants, especially heavy metals. Ingestion of these contaminants by animals causes deposition of residues in meat (Sabir *et al*., 2003).

The main sources of heavy metal contamination are growing and are represented, especially, by pesticides, fertilizers, industrial processes and exhaust gases from automobiles (Albu, 2010). The capacity of these metals to accumulate in living organisms. Lead, for example, bio-cumulates in plants and animals. Its concentration is generally magnified in the food chain (Halliwell *et al*., 2000). Cadmium has a long residence time in human tissues (10-40 years), especially in the kidneys (Rubio *et al*., 2006).

Exposures (through ingestion, inhalation, and injection) to nonessential trace metals, such as As, Cd, and Pb, can cause carcinogenic and non-carcinogenic adverse effects to human beings, even at low concentrations, while the essential ones can also be toxic when occurring at concentrations above the safety thresholds in food products (NHFPC and SFDA, 2017).

Heavy metals are known to disrupt important physiological processes in living cells, and have been responsible for various pathological conditions with possible contributions to cancer development as known cumulative poisons, there is the need for stringent regulatory control of these heavy metals in cured meat products imported into or produced indigenously in the country in order to minimize the risks to public health (Adejumo *et al.,* 2016). So many trials were carried out to control these elements and their serious problem, one of these trials is probiotics. Their mechanism of action in reduction of these toxic hazards may be attributed to that the metal ions bind to the cell wall and extracellular polysaccharides by viable or inactive cells including adsorption, ion exchange, complexation, chelation and microprecipitation (Veglio *et al.,* 1997).

E. faecium strains (*E. faecium* EF031 and *E. faecium* M74) are able to bind to lead and cadmium and may be used in the production of fermented functional foods, which will be healthy via its detoxification properties (Topcu and Bulat, 2010).

Therefore the present study was planned to detection of heavy metals in some meat products as beef burger, beef kofta, beef minced meat and beef sausage and comparing them with Egyptian standard and its biocontrol by the probiotic strain *enterococcus faecium* M74.

2. MATERIALS AND METHODS

2.1. Collection of Samples:

A grand total of 120 random samples of meat products represented by beef burger, beef sausage, beef minced meat and beef kofta (30 of each) were collected from different supermarkets located Cairo and Giza governorate and transferred to the laboratory in an insulated ice box.

2.2. Determination of heavy metals:

2.2.1. Washing procedures (AOAC, 2006).

2.2.2. Digestion technique (Tsoumboris and Papodoulou, 1994).

2.2.3. Preparation of blank and standard solutions (Shibamoto and Bjeldanes, 2000).

2.2.3. Analysis:

The digest, blanks and standard solutions were aspirated by Atomic Absorption Spectrophotometer (VARIAN, model AA240 FS, Australia) and analyzed for their concentrations of such elements. The apparatus has an auto sampler, digital absorbance and concentration readout which capable of operating at wavelength 217, 228.8, 193.7 for analysis of lead, cadmium and arsenic, respectively. The obtained results of heavy metals in the examined samples were calculated as mg/kg on wet weight (ppm).

2.3. Effect of *Enterococcus faecium (M74)* on concentration of lead and cadmium experimentally inoculated into minced meat:

The bacterial strain used in this study was probiotic strain of *E. faecium* M74 obtained from Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, Osaka, Japan. Cadmium and lead standard solution (1000 mg/L) in HNO_3 (2%, w/w) were obtained from Fluka (Sigma-Aldrich) and used to prepare the diluted solutions required for binding studies.

1. Preparation of bacterial suspension

Enterococcus faecium strain (M74) was cultivated in Brain Heart Infusion (BHI) Broth (Fluka, Sigma-Aldrich Chemie GmbH) for 24hr at 37ºC to prepare an overnight culture. One mL of the cultivated bacterial suspension was decimally diluted in sterile peptone water (0.1%, w/v) (Merck, Darmstadt, Germany). Accordingly, the viable count of *E. faecium* strain was carried out on BHI Agar (Fluka, Sigma-Aldrich Chemie GmbH) according to using plate count method (A volume of the culture broth corresponding to approximately 1×10^7) (Halttunen *et al.,* 2007).

2. Binding assay

The bacterial strain was suspended in 1 Kg minced meat. The mixture was adjusted to reach a final concentration of either 1×10^7 bacteria and 50 mg/Kg ionic lead standard solution and 1×10^7 bacteria and 10 mg/Kg ionic cadmium standard solution according to Halttunen *et al.* (2008) with some modification. Minced meat with bacterial stain and metal solution were incubated for 24h on a Finemixer SH2000 orbital shaker (FINEPCR, Seoul, Korea) with soft agitation. Minced meat contaminated with metals was served as a control assay, the test groups represented minced meat contaminated with heavy metals and *E. facium* were served as treated groups. The samples were acidified with ultrapure HNO3 and examined at zero, 8, 16 and 24 hour time points for measuring the free metal by flame atomic absorption spectrophotometer

3. RESULTS

3.1- Heavy metals residue in the examined meat products:

3.1.1. Lead:

Regarding to data obtained in table (1), it is indicated that the lead levels (ppm) in the examined meat product samples varied from 0.02 to 0.26 with an average of 0.13 ± 0.01 for beef minced meat, 0.05 to 0.51with an average 0.24 ± 0.01for beef kofta, 0.01to 0.48 with an average of 0.21 ± 0.01 for beef burger and 0.08 to 0.88 with an average of 0.36 ± 0.02 for beef sausage. Also, table (1) clarified the incidence of lead in the examined meat products, the detectable positive samples were 18 samples (60%), 20 samples (66.7%), 20 samples (66.7%) and 22 samples (73.3%) of the examined beef minced meat, beef kofta, beef burger and beef sausage, respectively.

In addition, according to the safe permissible limit stipulated by EOS (2010) for lead in meat products which is 0.1 ppm, it was indicated that the percentage of unaccepted samples were 26.7% (8 samples), 46.7% (14 samples), 46.7% (14 samples) and 53.3% (16 samples) for minced meat, kofta, beef burger and sausage, respectively (table, 2).

3.1.2. Cadmium:

Table (3) revealed that the cadmium levels (ppm) in the examined meat product samples varied from 0.01to 0.26 with an average of 0.07 ± 0.01 for beef minced meat, 0.01to 0.33 with an average 0.11 ± 0.01 for beef kofta, 0.01to 0.57 with an average of 0.18 ± 0.01for beef burger and 0.04 to 0.39 with an average of 0.16 ± 0.02 for beef sausage. Furthermore, our results revealed that the percentage of occurrence of cadmium in the examined meat products samples were 40% (12samples), 40% (12samples), 46.7% (14samples) and 60% (18 samples) of the examined beef minced meat, beef kofta, beef burger and beef sausage, respectively table (3).

According to the safe permissible limit stipulated by EOS (2010) for cadmium in meat products which is 0.05 ppm, it was indicated that the percentage of unaccepted samples were 26.7% (8 samples), 33.3% (10 samples), 33.3% (10 samples) and 46.7% (14 samples) for beef minced meat, beef kofta, beef burger and beef sausage, respectively(table, 4).

3.1.3. Arsenic:

Regarding to data obtained in table (5), it is indicated that the arsenic levels (ppm) in the examined meat product samples varied from 0.03 to 0.05 with an average of 0.04 ± 0.01 for beef minced meat, 0.01to 0.07 with an average 0.04 ± 0.01 for beef kofta and 0.06 to 0.15 with an average of 0.10 ± 0.02 for beef sausage. Also, table (5) clarified the incidence of arsenic in the examined meat products, the detectable positive samples were 4 samples (13.3%), 8 samples (26.7%) and 8 samples (26.7%) of the examined minced meat, kofta and sausage, respectively. Arsenic not detected in all examined minced meat samples. According to the safe permissible limit stipulated by Global Agricultural Information Network "GAIN" (2014) and (Codex, 1995) for arsenic in meat products which is 0.5 ppm, it was indicated that all of the positive samples were within permissible limits.

3.2. Effect of *Enterococcus faecium (M74)* on concentration of lead and cadmium experimentally inoculated into minced meat:

The results obtained in table (7) revealed that the *E. faecium* strain removed lead efficiently from the minced meat. Lead content (50 ppm) in the group treated with *E. facieum* (M74) showed reduction after 8, 16 and 24 hrs of incubation, their reduction rate were 66.4%, 73% and 82.6%, respectively.

Table (5) showed that the *E. faecium* (M74) strain removed cadmium efficiently from the minced meat. cadmium content (10ppm) in the group treated with *E. facieum* (M74) showed reduction after 8, 16 and 24 hrs of incubation, their reduction rate was 59%, 67% and 71%, respectively.

Table (1): Statistical analysis of lead levels (ppm) in the examined samples of meat products (n=30).

Meat products	+ve samples		Min	Max	Mean ± S.E
	No	%			
Minced meat	18	60	0.02	0.26	0.13 ± 0.01 [c]
Kofta	20	66.7	0.05	0.51	0.24 ± 0.01 [b]
Beef burger	20	66.7	0.01	0.48	0.21 ± 0.01 [b]
Sausage	22	73.3	0.08	0.88	0.36 ± 0.02 [a]

Table (2): Acceptability of the examined meat products according to their lead levels contents (n=30).

Products	Lead (ppm)	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Minced meat	< 0.1	22	73.3	8	26.7
Kofta	< 0.1	16	53.3	14	46.7
Beef burger	< 0.1	16	53.3	14	46.7
Sausage	< 0.1	14	46.7	16	53.3

* Egyptian Organization for Standardization "EOS" (2010).

Table (3): Statistical analysis of cadmium levels (mg/Kg) in the examined samples of meat products (n=30).

Meat products	+ve samples No	+ve samples %	Min	Max	Mean ± S.E
Minced meat	12	40	0.01	0.15	0.07 ± 0.01 [b]
Kofta	12	40	0.01	0.33	0.11 ± 0.01 [b]
Beef burger	14	46.7	0.01	0.57	0.18 ± 0.01 [a]
Sausage	18	60	0.04	0.39	0.16 ± 0.02 [a]

Table (4): Acceptability of the examined meat products according to their cadmium levels contents (n=30).

Products	Cadmium (mg/Kg)	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Minced meat	< 0.05	22	73.3	8	26.7
Kofta	< 0.05	20	66.7	10	33.3
Beef burger	< 0.05	20	66.7	10	33.3
Sausage	< 0.05	16	53.3	14	46.7

* Egyptian Organization for Standardization "EOS" (2010).

Table (5): Statistical analysis of arsenic levels (mg/Kg) in the examined samples of meat products (n=30).

Meat products	+ve samples No	+ve samples %	Min	Max	Mean ± S.E
Minced meat	4	13.3	0.03	0.05	0.04 ± 0.01 [b]
Kofta	8	26.7	0.01	0.07	0.04 ± 0.01 [b]
Beef burger	0	0	-	-	-
Sausage	8	26.7	0.06	0.15	0.10 ± 0.02 [a]

Table (6): Acceptability of the examined meat products according to their arsenic levels contents (n=30).

Products	Arsenic (mg/Kg)	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Minced meat	< 0.5	30	100	0	0
Kofta	< 0.5	30	100	0	0
Beef burger	< 0.5	30	100	0	0
Sausage	< 0.5	30	100	0	0

*□□ (GAIN, 2014)

Table (7): Effect of *E. faecium* culture ($1x10^7$) on the levels of lead experimentally inoculated to minced meat (50 mg/Kg).

Group / Storage time	Control (mg/L)	*E. faecium* Treated group (mg/L)	Reduction %
Zero time	50	50	-------
8 hours	50	16.8	66.4
16 hours	50	13.5	73.0
24 hours	50	8.7	82.6

Table (8): Effect of *E. faecium* culture (M74) ($1x10^7$) on the levels of cadmium experimentally inoculated to minced meat (10 mg/Kg).

Group / Storage time	Control (mg/L)	*E. faecium* Treated group (mg/L)	Reduction %
Zero time	10	10	-------
8 hours	10	4.1	59.0
16 hours	10	3.3	67.0
24 hours	10	2.9	71.0

4. DISCUSSION

4.1 Heavy metal residues in the examined meat products:

4.1.1. Lead:

Regarding to data obtained in table (1), For the examined beef minced meat, nearly similar results were reported by Demirezen and Urue (2006) (0.115ppm), while higher records were reported by Abd El- Dayem and Abou El-Magd(2004) (0.748 ppm). Higher results were reported by Abd El- Dayem and Abou El-Magd(2004) (1.520 ppm) for beef burger. Regarding the recorded results of sausage samples, lower results were recorded by Ji-Hun Jung *et al.* (1999)

(0.15±0.34), while higher records were reported by Abd El- Dayem and Abou El-Magd(2004) (1.250 ppm) and Zahran and Hendy (2015) (1.809± 0.16ppm).

Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults. The high level of lead may be attributed to the wide spread of environmental contamination (Dutta *et al.,* 2005), air borne traces, water pollution and contaminated fumes and effluents emitted from traffic (Shaheen *et al.,* 2005). The most sensitive targets for lead toxicity are the developing nervous, hematological, gastrointestinal, renal, reproductive and cardiovascular systems. Excretion of lead is primarily via the kidneys and can also be excreted with bile through the gastrointestinal tract, and half-life of lead in blood is about 30 days and 20-30 years in bone which contains up to 94% of the body burden of lead (patocka and Cerný, 2003).

4.1.2. Cadmium:

It is evident from the results recorded in Table (2), for the examined beef minced meat, nearly similar results were reported by Abd El- Dayem and Abou El-Magd(2004) (0.082ppm), while lower results were recorded by Demirezen and Urue (2006) (0.0079ppm). Higher results for the examined beef burger were reported by Abd El- Dayem and Abou El-Magd(2004) (0.983ppm). Moreover, nearly similar results reported by Abd El- Dayem and Abou El-Magd (2004) (0.153ppm), while lower results were obtained by Ji-Hun Jung *et al.* (1999) (0.08±0.05 ppm) and lower results recorded by Zahran and Hendy (2015) (0.114± 0.08) for the examined sausage samples.

The toxic effects of cadmium are noticeable in various ways. It can interfere with some of the organism's enzymatic reactions, substituting zinc and other metals, manifesting its action in several pathological processes such as renal dysfunctions, hypertension, arteriosclerosis, inhibition of growth, damages in the nervous system, bone demineralisation and endocrine disruption (Lafuente *et al.,* 2004).

4.1.3. Arsenic:

The obtained results were lower than that recorded by Zahran and Hendy (2015) (0.95± 0.15 ppm) for meat samples and 0.199± 0.09 ppm for sausage samples. Arsenic is widely distributed in nature, being found in food, the soil, water and airborne particles; it derives from both natural and human activities (Tchounwou, 1999).

4.2. Effect of *Enterococcus faecium (M74)* on concentration of lead and cadmium experimentally inoculated into minced meat:

The toxic nature of heavy metals (bioaccumulation and bio magnification) in food chain, so the contamination of the environment with these serious pollutants, related directly to public health (Hussain *et al.,* 2012).The cadmium and lead are reported amongst the top ten toxic metals in the Priority List of Hazardous Substances (ATSDR, 2007) for causing a grossly biological impact by bioconcentration, bioaccumulation phenomena. So many trials were carried out to control these elements and their serious problem, one of these trials is probiotics. Certain species of lactic acid bacteria (LAB), as well as other microorganisms, can bind metal ions to their cells surface or transport and store them inside the cell. Due to this fact, over the past few years interactions of metal ions with LAB have been intensively investigated in order to develop the usage of these bacteria in new biotechnology processes in addition to their health and probiotic. Preliminary studies in model aqueous solutions yielded LAB with high absorption potential for toxic and essential metal ions, which can be used for improving food safety and quality (Mrvčić *et al.,* 2012).

The obtained results were nearly similar to that reported by (Topcu and Bulat, 2010) they found that *E. faecium* M74 removed 42.9% to 93.1% of lead while the results for cadmium (reduction rate 53.5% to 91% of cadmium) were higher than the obtained result. Also, Hussien and Nosir (2017) used a probiotic strain (*Enterococcus.facium*(E980)) with lead and cadmium and the reduction rate ranging between 79.4 to 82.8% and 72.0 to 75.0% in lead and cadmium, respectively.

So concluded that *E. facieum* (M74) has higher metal binding ability to lead and cadmium. Moreover the binding ability of *E. facieum* (M74) to lead is higher than cadmium.

5. RECOMMENDATION:

Proper Collection and disposal of wastes should be as soon as possible. The risk of contamination from rubbish, sewage and other chemicals, should be avoided or reduced by using of stationary sale points located in a place away from these risks. Several experimental works on biocontrol of heavy metals by probiotic strains of lactic acid bacteria to use it in large scale.

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