



Detection of heavy metals and antibiotic compounds in some local and export meats

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Abstract

Chicken meat is one most common kind of poultry food used in the Saudi Arabia, where contamination of meat and meat products is a significant concern for public health. Meat can be contaminated by variety of contaminates in which the principle chemical contaminants include heavy metals and antibiotics. Especially during Hajj season the contamination of chicken meat is more likely to happen due to extensive demand and use of chemicals and antibiotics in chicken. Therefore, there is a need to assessed the complete profile of existing meat sources (local and export quality) in KSA for metal and antibiotic contamination. The present study was aimed to detect and measure various heavy metals and antibiotics in two types of meat which includes local chicken meat and export chicken meat marketed in Saudi Arabia. The sampling for assessment was conducted at two different times during Haj and after Haj. About 20 samples for each type of chicken were taken and the meat samples were analyzed for determination of heavy metals and antibiotics compounds in poultry meat. The results indicated that samples were completely clear of most of antibiotics. Only one meat sample was found contaminated with high values of antibiotic residues. During hajj both local and export meat showed trace metal concentrations higher than the permissible limits. Therefore, the consumption of unsafe meat during the peak time of tourism is a serious threat to public health.

Keywords: Chicken meat, heavy metals and antibiotics, Haj, trace metal concentrations.

Introduction

Chicken meat is one most common kind of poultry food used in the world. Contamination of meat and meat products is a significant concern for public health, meanwhile it can lead to illness of human due to inattention in the handling, treatment and storage of meat.

Meat and meat products may be contaminated by variety of contaminates in which the principle contaminants include heavy metals, antibiotics, microorganisms, metabolites, mycotoxins, hormones, nitrates, nitrites, pesticide residues, dioxins, , toxic pigments, and melamine etc.

Among principle contaminants in meat heavy metals is a growing concern which release due to the discharge of domestic sewage and industrial wastewhich may cause adverse impacts on human health. These effluents mostly contain high levels of heavy metals that accumulate in the form of macro particles and travel through many food chains. In food stuffs heavy metal traces can occur as residues due to their processing, storage and marketing (Hu et al., 2017). It can also be contaminated during their process, storage and marketing (Nuapia et al. 2018). The non-essential elements such as cadmium, lead, and mercury have high toxicity and a tendency to accumulate in meat.

For example, lead in the meat is taken up through the human digestive tract, which interferes with hemoglobin synthesis in the body and may inhibit several enzymes. Moreover, the heavy metals can be responsible for several damages in biochemical processes, leading to bones, kidney, brain, liver, the nervous and problems I cardiovascular system (Ekhatior et al., 2017).

Antibiotics are the chemicals that have been developed for protection of people and livestock from infections of bacteria (Hyung et al., 2017). Antibiotics are one of the highly used veterinary drugs which are authorized for treatment or prevent animal from diseases, however, this may also be illegally administered as a growth promoter drug.

The present study was aimed to detection of two main contaminants in the meat i.e. heavy metals and antibiotics in two types of meat which includes local chicken meat and export chicken meat. The sampling for assessment was conducted at two different times before Haj and after Haj. About 20 samples for each were taken and the meat samples were analyzed for determination of heavy metals and antibiotics compounds in poultry meat.

Materials and Methods

Chemicals

The chemical reagent s used in the present study includes Methanol , Merck, Acetone Merck, Acetonitrile Merck, *N,N*-Dimethylformamide (DMF) Merck, Formic acid Merck , Hydrochloric acid 32% Merck, Sodium hydroxid pellets Merck, di-Nitromphosphat, Citronsyre monohydrat Merck, Na₂HPO₄ Merck, Sulfadiazine Sigma, Sulfaquinoxalin Pestanal Fluka, Sulfamerazin Sigma, Sulfachlorpyridazin Sigma, Sulfamethizol Sigma, Sulfathiazol Sigma, Sulfamethoxazol Sigma. The standards for antibiotic detection include Sulfadiazine, Sulfamerazine, Sulfapyridine, Sulfathiazole, Sulfadimethoxine, Sulfamethazine, Sulfamethizole, Sulfamethoxazole, Sulfaquinoxaline, Tetracycline, Chloro-Tetracycline, Doxycycline, Oxy- Tetracycline, epi-Tetracycline, epi-Chloro-Tetracycline and epi-Oxy- Tetracycline. For the detection of metals CRM (certified refrence material) CHILI was used (certified for the estimation of B, Cr, Mn, Fe, Fe, Ni, Cu, Zn, As, Se, Cd, Sn, Ba, Hg, Pb and U).

Sampling of chicken meat

The chicken meat samples were taken from the local market and superstores located in the city of Jeddah. Saudi Arabia. Two types of meat samples were taken one is for local companies which produce chicken meat with in Saudi Arabia, and second is for export quality meat. The samples were taken in two time frames i.e. during Hajj and After Hajj. Total of 81 samples were collected of which 40 corresponds local meat samples where the rest (41 samples) were from export meat. In general about 20 samples belong to each category that is during hajj local meat, after hajj local meat, during hajj export meat and after hajj export meat. The samples were initially stored in a low cool storage unit (refrigerator) at around 0-1 °C.

Analytical Procedure

Determination of trace elements

The determination of trace elements in chicken meat samples was conducted by inductively coupled plasma mass spectrometry (ICP-MS). For sample preparation, we used a procedure described by other authors (Batista et al., 2012) and modified as discussed below. The estimated values were compared with international standards for metals in meat (WHO, 2000; FAO, 2000)

Sample Preparation

Pressure digestion of the meat samples were carried out. Meat samples (0.2-0.5 g) were weighed into a digestion vessel. Reagent blanks were prepared by addition of Milli-Q™ water in place of the sample. For each digestion batch (8 vessels), one reagent blank was prepared to monitor the background concentration of studied elements. A volume of 5 mL of concentrated HNO₃ and 2 mL of non-stabilized 30% H₂O₂ solution was added to each digestion vessel. Samples were left for approximately 1 h (pre-digestion) in the fume hood, then sealed, placed in the microwave, and digested using the program described in Table 3.1. Afterwards they were cooled, transferred quantitatively into acid-washed polypropylene tubes, diluted to the final volume (30 mL) with Milli-Q water, and stored at 6±4°C in the dark until analysis. Reagent blanks were prepared under the same conditions. For the samples where the digested samples were not clear sonication was carried out.

Analysis through ICP-MS

Quality control samples were run for the quality assurance of the samples. The initial reagent blank was run at first followed by fresh calibration standard and solvent blank. The meat samples were run on Luc-MS after standard calibration curve achieved 99% efficiency. After every 10 samples the solvent blank were tested to ensure the quality control of the samples. The ICP-MS was set up according to the procedure outlined in operation, calibration, maintenance SOP. After initial 20 minutes of initial warmup of the instrument the samples were tested through ICP-MS. All the samples were run in three replicates and the integration peaks represented the mean of all three replicates.

Determination of antibiotics in meat using LC-MS/MS

The antibiotics in meat were analysed by using DVFA (2007) method ANA-standard operating and analysis procedure (SOP) by Denmark was followed for the analysis of residual antibiotics in the chicken meat (SOP, Denmark)

Preparation of spiked samples as reference

The quality control of the extracted samples was ensured using a 50 ppb mixture of 32 different antibiotics was used. Working solution of 50 ppb was used to contaminate the samples to ensure quality control. The spiked samples were finally run on LC-MS following the same procedure as given for the rest of the samples.

Extraction of Samples

For the extraction of samples for antibiotics estimation each sample was weighed 1.00 g (1.00 ± 0.05 g for confirmation) in 15 mL plastic tube. To the sample 10 μ L standard solution was added along with 1000 μ L of deionized water using glass syringes. 5 mL of acetonitrile was added and sample were agitated for through mixing. Following the similar procedure a blank was also prepared without any samples. All the samples, blank and standards were placed on rotator for 10 mins. All the samples were individually centrifuged at 5000 rpm at 4°C for 10 minutes.

The supernatant were transferred to 4.0 mL peak bottomed centrifugation tube. Samples were dried under N₂ stream at maximum 50°C. dried samples

were re-dissolved in 1000 μ L 15% H₂O and MeOH under continuous shaking of 30 sec. Final extraction from the re-dissolved samples was carried out by centrifuging the samples at 13000 RPM, 4°C for 5 minutes. Centrifuged samples were transferred 200 μ L to HPLC vials and were analyzed using LC-MS.

Results and Discussion

Determination of heavy metals and antibiotics in the chicken meat of two types that is local and imported meat was carried out during and after hajj. The estimated amount of different metals is given below.

Detection of heavy metals in the chicken meat

The presence of different metals detected in the meat is presented in table 1. The estimation results indicated that almost all types of the meat samples contain certain amount of metals or heavy metals in them, all the samples were found to be contaminated with highly toxic metals like Cu, Co, Ni, Zn, Cd, Hg and Pb. There is no significant difference in the detection of metals among local and imported meats. This signifies that the contamination of metals to the meat could occur in any field of supply chain. The detection of metals in all types of meat indicated that using meal contaminated meat could attribute serious health concerns in human body. Previous studies also indicated that heavy metals can easily enter food chain through water, soil or even feed and their intake above safe limit could be related to detrimental health effects in human (Hooda et al., 1997). The researchers have highly focused the bioaccumulation impact of heavy metals and their related toxic effects (Bernard, 2008). Where some heavy metals are so toxic that even their presence in low concentration have potential to produce toxicity in human body (Nordberg et al., 2010). Presence of different metals during hajj is of serious concern as hajj is one of the prime tourism activity in Saudi Arabia. And presence of metals in chicken meat could pose serious health risks. The complete profile of the metals detected in the meat samples the metals like mercury, lead, zinc, chromium, copper, cadmium, nickel, iron and arsenic are of prime concern due to their related toxicity, which has been identified by different researchers. Table 1 revealed that chromium, manganese, iron, nickel copper, zinc, arsenic, cadmium and lead is present in all type of meat both in export and local quality. All the investigated metals were present in both types of meat that is local and export and were also at both sampling time i.e present both during hajj and after hajj samples.

Table Error! No text of specified style in document.1 Detection of metals in different chicken meat samples.

Metal Name	Local Meat		Export Meat	
	Detection During Hajj	Detection After Hajj	Detection During Hajj	Detection After Hajj
B	-	+	+	+
Ti	-	-	+	-
Cr	+	+	+	+
Mn	+	+	+	+
Fe	+	+	+	+
Ni	+	+	+	+
Cu	+	+	+	+
Zn	+	+	+	+
As	+	+	+	+
Se	+	+	+	+
Cd	+	+	+	+
Sn	+	+	+	+
Ba	+	+	+	+
Hg	-	+	+	-
Pb	+	+	+	+
U	+	+	+	+

Detection of antibiotics in the chicken meat

The detection of different antibiotics in the spiked samples. The concentration of the spiked samples were found convincing for the quality assurance of the method and sample analysis through LC-MS. Table 2 represented the detection of different antibiotics in the export chicken meat samples at different time frames (Sample A: pre hajj & sample B post hajj). The results indicated that no residues of any antibiotic

is present in the chicken meat irrespective of the sampling time. The absence of any form of antibiotic in the meat signifies its safety for human consumption and ensure associated risk reduction. The detection of antibiotics in the local chicken meat. The results indicated that samples were completely clear of most of antibiotics. However, traces of Doxycyclin 1 & 2 are present in pre-hajj samples of the meat. Where in after hajj samples Oxy- Tetracycline 1 & 2 is also present alongwith the Doxycyclin 1 & 2.

Table2: Detection of antibiotics in different chicken meat samples.

Antibiotic Name	Local Meat		Export Meat	
	During hajj	After hajj	During hajj	After hajj
Sulfadiazine 1	-	-	-	-
Sulfadiazine 2	-	-	-	-
Sulfamerazine 1	-	-	-	-
Sulfamerazine 2	-	-	-	-
Sulfapyridine 1	-	-	-	-
Sulfapyridine 2	-	-	-	-
Sulfathiazole 1	-	-	-	-
Sulfathiazole 2	-	-	-	-
Sulfadimethoxine 1	-	-	-	-
Sulfadimethoxine 2	-	-	-	-
Sulfamethazine 1	-	-	-	-
Sulsamethazine 2	-	-	-	-
Sulfamethizole 1	-	-	-	-
Sulfamethizole 2	-	-	-	-
Sulfamethoxazole 1	-	-	-	-
Sulfamethoxazole 2	-	-	-	-
Sulfaquinoxaline 1	-	-	-	-
Sulfaquinoxaline 2	-	-	-	-
Tetracycline 1	-	-	-	-
Tetracycline 2	-	-	-	-
Chloro-Tetracycline 1	-	-	-	-
Chloro-Tetracycline 2	-	-	-	-
Doxycycline 1	+	+	-	-
Doxycycline 2	+	+	-	-
Oxy- Tetracycline 1	-	+	-	-
Oxy- Tetracycline 2	-	+	-	-
epi-Tetracycline 1	-	-	-	-
epi-Tetracycline 2	-	-	-	-
epi-Chloro-Tetracycline 1	-	-	-	-
epi-Chloro-Tetracycline 2	-	-	-	-
epi-Oxy- Tetracycline 1	-	-	-	-
epi-Oxy- Tetracycline 2	-	-	-	-

Detection of doxycycline during hajj

The Figure1 represents the detection of Doxycycline 1 & 2 which were only present in local meat during hajj samples. The results also indicated that only 6 out of 22 analysed samples were contaminated with Doxycycline 1 & 2. The presence of Docycyline in the

meat is an indication of antibiotic contamination in the meat which may lead to bacterial or microbial resistance. Bacterial resistance to different medicinces is already forcing use of antibiotics to the post-antibiotic era. Such contamination and related resistance can be transferred to the human biome by using contaminated meat (Marshall and Levy, 2011).

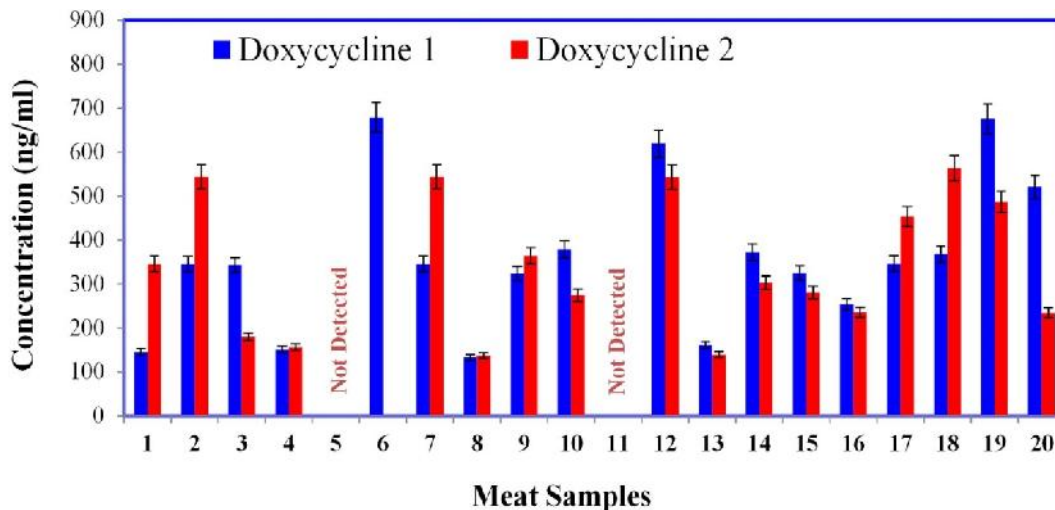


Fig. 1. Determination of antibiotics in local chicken meat during hajj samples.

Detection of doxycycline after hajj

Similarly the Fig. 2 represents that not only Doxycycline 1 & 2 is present in post hajj local meat samples but also another antibiotic Oxy- Tetracycline 1 & 2 were detected in one sample that further raised the concern about the residual effects of antibiotics in meat. The frequency of occurrence of doxycycline 1 is also greater than pre hajj samples that is 13/22 where doxycycline 2 was also detected in 12 out of 22 samples analyzed for antibiotic contamination in the chicken meat. Contamination of Oxy- Tetracycline 1

& 2 was only detected in one sample of the local chicken after hajj meat sample. Previously chicken meat was also found contaminated with different antibiotics (Courrol and Samad, 2018). In another study poultry meat was found contaminated with different residues of antibiotics (Wang et al., 2017). The contamination of meat with the antibiotics is attributable to the administration of drugs to increase productivity by enhancing disease tolerance in different chicken varieties (Centner, 2016)

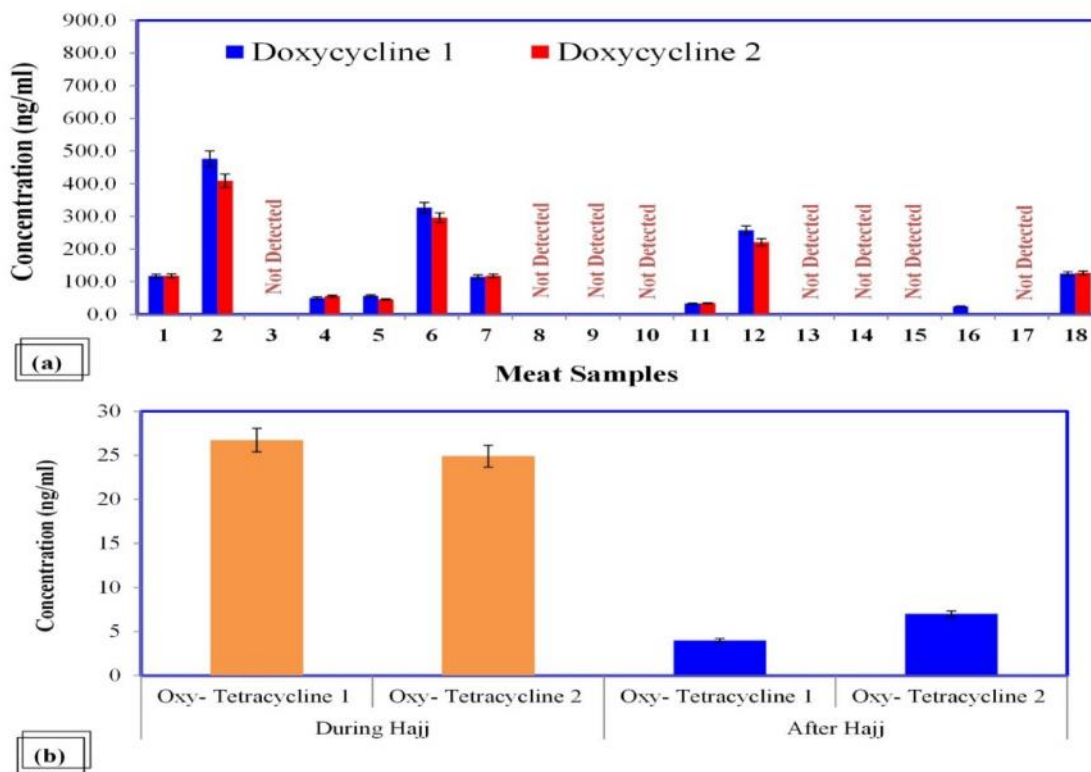


Fig. Error! No text of specified style in document.2 Determination of antibiotics in local chicken meat Post hajj samples (a) Detection of Doxycycline 1 and Doxycycline 2 in all samples, (b) Detection of Oxy-Tetracycline 1 and Oxy-Tetracycline 2

Conclusion


The study presented a complete profile of metals and antibiotics in the chicken meat. This data derived from could be used for risk analysis of heavy metals and antibiotics in human food origin. The higher concentration of different metals like Cd, Hg, Cu, Zn and As in the during hajj samples signifies the importance of quality assurance during the peak consumption time. The fact that during hajj the demand of the chicken meat increases many folds and fulfill that demand many local and foreign companies came on ground. Therefore, the consumption of unsafe meat during the peak time of tourism is a serious threat to public health. In few samples the concentration of Cr, and Pb were found higher than safer limits therefore present a risk of metal toxicity in humans. Similarly the DI values based on current estimations also indicate serious public health concerns. The results highlight the significance of the continuous monitoring of these metals in chicken feed, water and concurrently meat and eggs because they can harmful when they exceed the permissible limits for food and once transferred to the human body through consumption of contaminated meat. It would be advisable to form safe limits for consumption on daily use bases for heavy metals in meat and eggs. The results also indicated the need of total risk assessment from exposure to cumulative metals in meat that should be concerning. Consuming the foods polluted with heavy metals has varying negative impacts on human health. The results of present study revealed the presence of different concentrations of heavy metals in chicken meat marketed in Saudi Arabia. Furthermore, the concentrations of some metals were above the safe limits thus might be a concern for the consumers.

The study also comprehensively screened the antibiotic residues in chicken meat at during hajj and post hajj time in both local and export meat. Antibiotics were residues were detected in few samples only. Only few meat sample was found contaminated with high values of antibiotic residues. However the presence of remains of doxy-cyline and oxy-tetracycline in the local meat samples were only present in during hajj samples. These findings showed a serious concern of antibiotic residues in chicken meat that might pose a health risk on population.

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