Egypt. J. Chem. Environ. Health, 2 (2):598-610 (2016)
On line ISSN: 2536-9164.

Experimental study on the effect of different cooking method of oxytetracycline residues in chicken meat

Nashwa , M. Zaki *; Arwa , H. Nassar *; Saleh , Shafik* And Nahla , S.L. Elshater**

*Food Hygiene Dept., Animal Health Research Institute , Mansoura, Egypt & ** Reference Lab for Quality Control on Poultry Production , Animal Health Research Institute , Dokki – Giza.

Abstract

Tetracyclines (TCs) are licensed for use in a wide range of livestock species including chicken, and can be administrated either orally or by injection , resulted in the presence of TCs residues in their edible tissues, intended for human consumption, causing a health threat. Hence, the stability of TCs residues in chicken tissues under cooking conditions is an important research area. The present study was conducted to determine the effect of different cooking process like boiling, roasting and microwave on muscle tissue and liver of 40 broiler chicken which experimentally injected by oxtetracycline for 3 successive days and analyzed by HPLC. The obtained results showed a reduction in concentration of oxytetracycline residues after different cooking process. Microwave process showed the highest reduction effect on TCs residues followed by boiling and roasting finally process with average mean values of 1032±405, 2392±1039 and 3297±1357*ng /g* for breast respectively with reduction percentage of 75.4%, 42.3%, and 31.4%for microwave, boiling, and roasting, respectively. While the average mean values for thigh (2142±899, 4585±1699, and 6592±2335 *ng /g* for microwave, boiling, and roasting, respectively) moreover , Liver samples recorded 8473±4031, for boiling and 6975±2079 *ng /g* for roasting. The results show significant reduction ($p<0.05$).in the antibiotic residues after both boiling ,roasting and microwave processes for oxytetracycline residues. in this study microwaving had a great effect on reducing OTC residues. Generally, sufficient cooking temperature and time can have a significant effect on the reduction of TCs residues and provides an additional margin of safety for consumers.

Introduction

Antibiotics are used in chicken not only for treatment of diseases but also to maintain health and promote their growth, but lack of proper application and handling can lead to presence of antibiotic residues in food of animal origin particularly meat and egg. Chicken treated with antibiotics are required to be held for specific withdrawal period of time before slaughtering. Otherwise, presence of antibiotic residues in animal tissues could be constitute an adverse effects on human health.. TCs are licensed for use in a wide range of livestock species including chicken, and can be administrated either orally or by injection.

Chicken meat consists of high-quality protein that contains a high variety of essential amino acids as well as a relatively low amount of fat. In addition, fat in chicken is mostly of the unsaturated type, which protects against heart diseases. Thus, chicken meat is often recommended by nutrition counselors as an alternative to other types of red meat, which typically has more fat and more saturated fatty acids. Chicken meat provides not only high- quality protein, but also important vitamines and minerals. In intensive chicken production, the main purpose of using antibiotics and veterinary drugs, is a protective treatment for prevention of the occurrence of disease and to safeguard the health of consumer and welfare of animal **(Cannavan, 2004)**. Drug residues will affects the quality palatability, aroma of meat through increase protein deposition, usually linked to fat utilization that decrease fat content in the carcass and increase meat leanness **(Aliu, 2004 and Shanker *et al.*, 2010).** The existence of Tetracycline (TCs) residues in chicken products will be considered of public health hazard and subsequently it will have an adverse effect on consumer health as well as presence of bacterial resistance, allergic reactions, toxicity, carcinogenic effects and disturbance of natural intestinal micro-flora. Therefore, the maximum residual limits (MRLs) of TCs recommended by **FAO/WHO Expert Committee on Food Additives (2004)** were 200 µg/kg and 600 µg/kg for chicken tissues and liver, respectively. In addition, the **European Commission (EU, 2010)** had set the MRLs of TCs of 100 µg/kg and 300 µg/kg for chicken tissues and liver, respectively. However, TC levels above the MRLs have been reported in chicken products in many countries such as Mexico (**Vásquez-Moreno *et al.*, 1990**) and Egypt (**Salama *et al*., 2011).** Such residues in the chicken meat may have a potentially dangerous health threat to the consumer life. Therefore, this work was aimed to determine the effect of cooking (Boiling, Roasting and Microwaving) on the residual level of Oxytetracyclines (OTC)in chicken meat through quantitative analysis using high performance liquid chromatography (HPLC).

Material and Methods

I. Chicken and drug administration:

In this experiment Forty broiler chicken about 2 kg which breeded without administration of any type of antibiotics were divided into 4 group (10 of each) and injected intramuscular with oxytetracycline *(*Oxytetracycline® 20%, Adwia*)*in thigh muscle by concentration (20mg/kg b.wt.) for 3 successive days. The chicken were slaughtered after 12 hours of the last injected dose then divided to 4 group***:***

1st group: was considered as control positive and the sample were taken from thigh, Breast muscle and liver.

2nd group: was boiled for 30 minute and the samples were taken from thigh Breast muscle and liver.

3rd group: was roasted for 40 minute and the samples were taken from thigh, Breast muscle and liver.

4rd group: was microwaved for 15 minute and the samples were taken from thigh , Breast muscle only

Chicken meat was separated from bone (deboning), chopped and re-alyzed to obtain the initial (0-time) OTC levels. For each chopped chicken meat sample, three portions (100 g each) were placed into heat-safe plastic bags and cooked as described in cooking procedures

II. Chemicals and Reagents

Standards of OTC supplied by **Sigma (St Louis, MO, USA)**. Methanol and acetonitrile were of HPLC grade. Trichloroacetic acid, citric acid monohydrate, sodium citrate trihydrate and oxalic acid were of analytical grade. Water was purified before use by a Milli-Qsystem (Millipore, Bedford, MA, USA).

III. Cooking Procedures

(1) **Boiling**

Slices of chicken meat (100 g each) were placed in boiling water and boiled for 30minutes. The cooked meat had a “well done” appearance on the outside..

(2) Roasting

From each chicken, thigh samples (100 g each) were grilled on coal. Sample was removed at 40 minute allowed to cool at room temperature, minced and blended with the resultant juice and analyzed in triplicate. The same procedure was applied to chicken breast samples (100 g) and liver sample.

(3) Microwaving

From each chicken, take thigh samples (100 g each) were placed in a microwave bag and cooked in a Gold-star Microwave Oven (Model ER-535 MD) (2450 MHz) at six power level. The samples were withdrawn after 15 min, allowed to cool at room temperature, minced and blended with the resultant juice and analyzed in triplicate. The same procedure was applied to chicken breast samples (100 g).

IV. Analytical Method

chromatograph (Agilent Technologies, Palo Alto, CA, USA) The determination of TC residues was achieved according to (**Shalaby et al. 2011)** using HPLC-DAD. The HPLCDAD system consisted of a HP 1100 equipped with an auto sampler, quaternary pump 4 and a diode array detector.A reversed-phase Nucleosil 100 C18 analytical column (250 mm × 4.6 mm i.d., 5 μm, Germany) was used. Briefly,TCs in samples were extracted by citrate buffer and trichloroacetic acid, followed by sonication and centrifugation. Purification was performed on SPE (Strata C18-E cartridge) using 10 mL of 0.01 M methanolic oxalic acid for the elution of TCs. Separation of TCs was conducted on a Nucleosil 100 C18 analytical column by multistep gradient elution, and monitored at the wavelength of 351 nm.

V. Analytical Quality Assurance

The analytical quality assurance of the TC residues method was performed using cooked chicken meat by spiking TCs into a negative-TCs cooked chicken thigh and breast samples at a level of 100 ng/g, to meet the MRLs of **(EU 2010)**. Spiked samples were left to stand at 4°C for 1 h before analysis to establish the accuracy and precision, limits of detection (LOD), limits of quantification (LOQ) and sensitivity of the method. The LOD is considered to be the quantity yielding a detector response approximately equal to thrice the background noise. The LOQ is the lowest amount that can be analyzed within acceptable precision and accuracy at signal to noise ratio of 10. Each sample was analyzed in triplicate and the mean was considered without correction for recovery

VI. Thermostability Parameters

The traditional model based on first-order kinetics was used to describe degradation curves **(Ramaswamy and Chen. 2004)**. Degradation curves were obtained for each cooking procedure. The pattern was expressed as an equation: {y = k 10mx, where, y was the tetracycline residues (ng/g) in a sample after cooking, k was a constant equal to the tetracycline residues (ng/g) in a sample at 0-time, m was the slope of the regression line, and x was the cooking time (min)}. Decimal reduction times, i. e. D-value (minutes of heating required to drop the antibiotic concentration by 10-fold or one log10 cycle) were calculated from the slope of the portion of the linear section of the degradation curves. The coefficients of determination (R2),95% confidence limits, and statistical differences (p = 0.01) were calculated by GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

VII. Statistical Analysis

The ANOVA test was performed for statistical analysis using Assistant computer programs**(Silva. and Azevedo 2006).** A p value of less than 0.05 was considered to be statistically significant.

Egypt. J. Chem. Environ. Health, 2 (2):598-610 (2016)
On line ISSN: 2536-9164.

Results and Discussion

Boiling procedures decrease OTC residues in chicken meat and liver. Moreover, antibiotics can be refractory for heat degradation in animal tissues unless high temperature levels are maintained for considerable period (**O´brine *et al.*, 1981)** This proved that the temperature and duration time resulted in disappearance of antibiotic residues in all edible tissues except some organs. However, some antibiotics are heat stable such as chloramphenicol **(Kan, 1995)**

While others are polymerized at higher temperature (200ºC) and produce toxic or mutagenic products ***(*Booth and Mcdonalid, 1988*).*** The presence of antibiotic residues after cooking represents serious problems for human beings consuming such tissues hyper sensitivity or ever toxicity and development of bacterial resistant **(Thomes, 1994 and Butay *et al*., 2001*)***

The results obtained in Table (1& 2) revealed that the maximum and minimum concentration of oxytetracycline in breast samples after12 hours slaughter time were 12124.2 and 988 ng/g, respectively, with an average mean of 4194±1677ng/g whereas after heat treatment by boiling , roasting and microwave maximum and minimum concentration of oxytetracycline was (7636.9, 570, 9843.1, 750, and 2954.2 , 299 ng/g) respectively with an averages 2392±1039, 3297±1357and 1032±405, respectively.)

Table (1): ***Mean values of oxytetracyclin HCL residues in experimentally injected chickens (ng /g) by HPLC before and after heat treatment*** **(n=10 of each)**

Sample type		Breast			Thigh			Liver		
		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Before treatment		4194± 1677	988	12124.2	11190± 3392	1042	36795	10026± 3041	4962.4	36795.3
After heat Treatment	Boiling	2392± 1039	570	7636.8	4585± 1699	668	18488	8473± 4031	2100	42691
	Roasting	3297± 1357	750	9843.1	6592± 2335	861	25217	6975± 2079	2723.0	25217
	Microwave	1032± 405	299	2954.2	2142± 899(	350	9658	NP	NP	NP

NP = (Not Performed)

Followed by boiling and finally Roasting. with reduction percentage of (42.3%,31.4% and 75.4%for boiling , roasting and microwave respectively), as shown in table (2) and figure (1, 5, 9 &11) this result nearly The results recorded in Table (2).figure (1) revealed that,**)** there were a highly significant difference between OTC residues in examined chicken breast samples before treatment and that after microwaving ($P<0.001$). Also, such difference was existed between Roasting and microwaving samples ($P<0.05$). Otherwise, in this study microwaving had a great effect on reducing OTC residues in examined chicken breast samples similar to that obtained by **Abou-Rayal *et al.*, (2012)** and **Fadwa, *et al.*, (2015)**

Table (2): mean Oxytetracycline residues in chicken breast before and after different thermal treatments & reduction percentage

Chicken breast (Mean ± SE) with reduction percentage						
Before treatment	After thermal treatments					
	Boiling		Roasting		Microwaving	
	Mean(ng/g)	R P %	Mean(ng/g)	R P %	Mean(ng/g)	R P %
4194±1677 [(a)]	2392±1039	42.3%	3297±1357 [(a)]	31.4%	1032±405 [(A)]	75.4%

A & a There were significant differences between the small and capital litter.

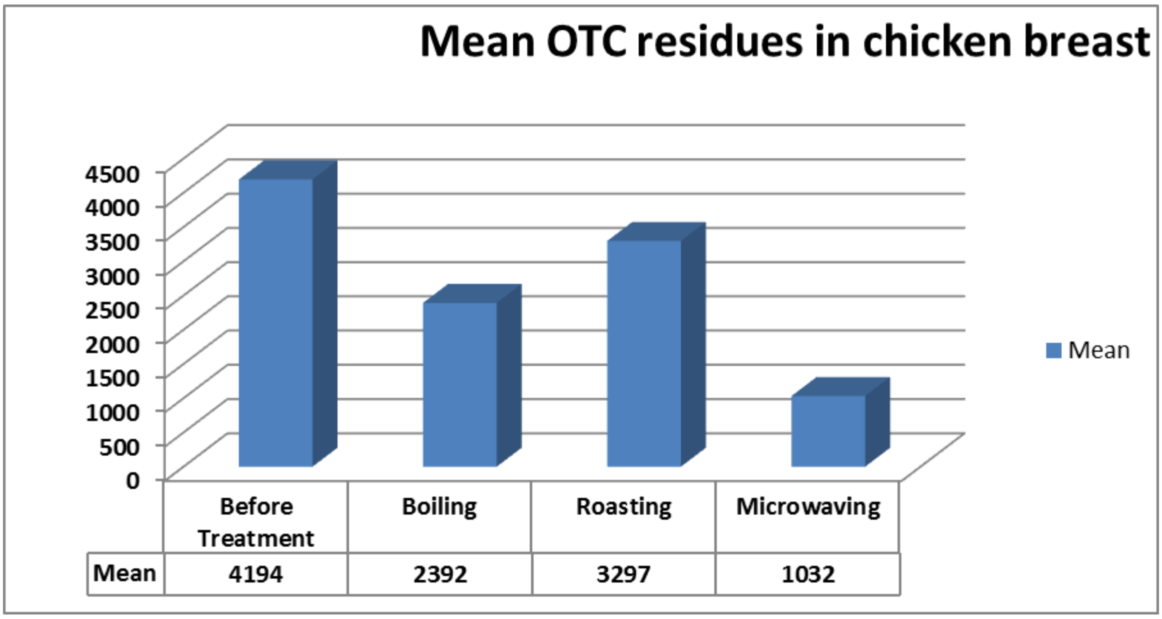

Fig. (1) Mean oxytetracycline residues in examined chicken breast samples

Moreover The results obtained in Table (1& 3) revealed that the maximum and minimum concentration of oxytetracycline in thigh samples after12 hours slaughter time were **36795** and **1042** ng/g, respectively, with an average mean of **11190±3392** ng/g whereas after heat treatment by boiling , roasting and microwave maximum and minimum concentration of oxytetracycline was (**18488**, **668**, **25217**, **861**, and **9658**, **350** ng/g) respectively with an averages **4585±1699**, **6592±2335**and **2142±899** respectively.

The obtained results from Table (3) revealed that, there were a highly significant difference between OTC residues in examined thigh samples before treatment and that after microwaving (P<0.001). Also, such difference was significant between boiled and microwave samples (P<0.05). Otherwise, in this study microwaving had a great effect on reducing OTC residues in examined chicken thigh samples followed by boiling and finally Roasting with reduction percentage of (**59%**,**41.1%** and **80.9%**for boiling , roasting and microwave respectively, as shown in table (3) and figure (2, 4, 6 & 8) This result nearly similar to that mentioned by **Abou-Rayal, *et al*. (2012*).*** On the contrary, **Al-Ghamdi *et al.* (2000)** stated that OTC was more stable than TTC, CTC and DOC in meat and liver samples after cooking by boiling.and agree with **Fadwa, *et al.*, (2015**) mention that Differences between oxytetracycline residues of raw and cooked meat samples in both cooking methods

Table (3): mean Oxytetracycline residues in chicken thigh before and after different thermal treatments & reduction percentage

Chicken thigh (Mean ± SE) & reduction percentage						
Before treatment	After thermal treatments					
	Boiling		Roasting		Microwaving	
	Mean(ng/g)	R P %	Mean(ng/g)	R P %	Mean(ng/g)	R P %
11190±3392(A)	4585±1699(a)	59%	6592±2335	41.1%	2142±899(a)	80.9%

A & a There were significant differences between the small and capital litter.

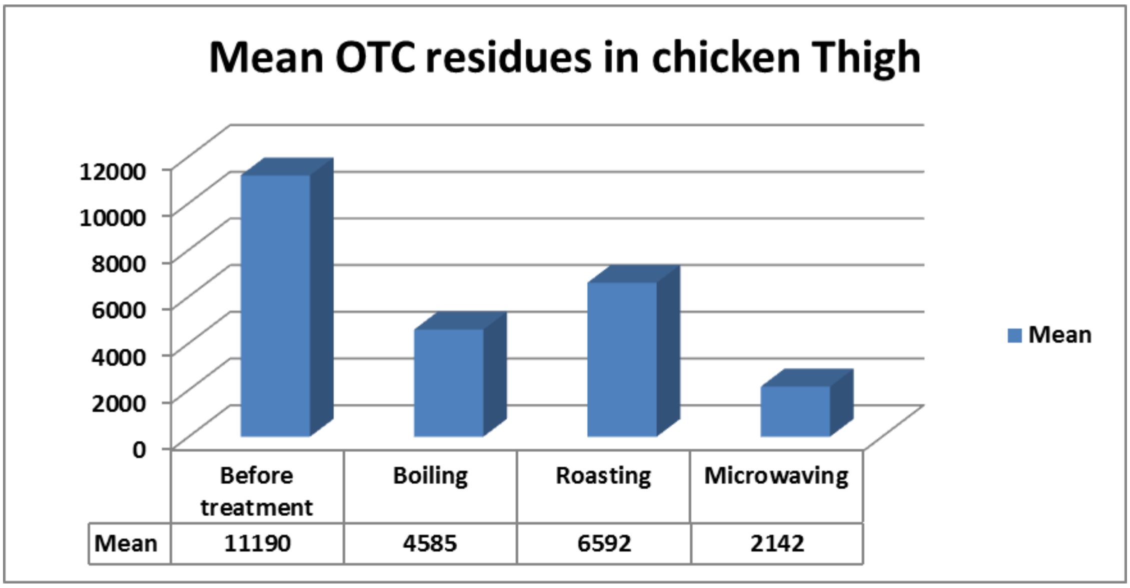

Fig. (2) Mean oxytetracycline residues in examined chicken thigh samples

Meanwhile The results obtained in Table (1& 4) revealed that the maximum and minimum concentration of oxytetracycline in liver samples after12 hours slaughter time were **36795.3** and **4962.4** ng/g, respectively, with an average mean of **10026±3041**ng/g whereas after heat treatment by boiling and roasting maximum and minimum concentration of oxytetracycline was (**42691**, **2100**, and **25217**, **2723** ng/g) respectively with an averages **8473±4031**and **6975±2079** respectively with reduction percentage of (15.5%and 30.4%for boiling and roasting respectively, shown in table (4) and figure (3 & 10). Results in Table (4) illustrated that there were no significant difference between different treatments of liver samples. This could be attributed to that OTC required high temperature for its degradation as in case of microwaving while normal temperature did not affect on the antibiotic residues The obtained data revealed that the reduction of TC residues in cooked samples was related to cooking procedures, cooking time and TC agents **(Javadi 2011)**. These findings were in agreement with those given by **Ibrahim and Moats (1994)** who stated that cooking of lamb meat by boiling (30 min). In this respect, reduced OTC by 95%, and microwave cooking (8 min) reduced the OTC content by 60%, while frying (8 min) led to17.3% reduction in the OTC content.this result agree with ***(*Abou-Rayal *et al.*, 2012 and Fadwa *et al.*, 2015)** Similar results with different values were recorded for TC residues. It was previously reported that prolonged cooking of meat might inactivate the antibiotics **(Moats, 1988).**

The overall loss of oxytetracycline residues was due to denaturation of protein **(Nguyen *et al.*, 2013**)

Egypt. J. Chem. Environ. Health, 2 (2):598-610 (2016)
On line ISSN: 2536-9164.

Table (4): mean Oxytetracycline residues in chicken liver before and after different thermal treatments & reduction percentage

Chicken liver (Mean ± SE) & reduction percentage				
Before treatment	After thermal treatments			
	Boiling		Roasting	
	Mean(ng/g)	R P %	Mean(ng/g)	R P %
10026±3041	8473±4031	15.5%	6975±2079	30.4%

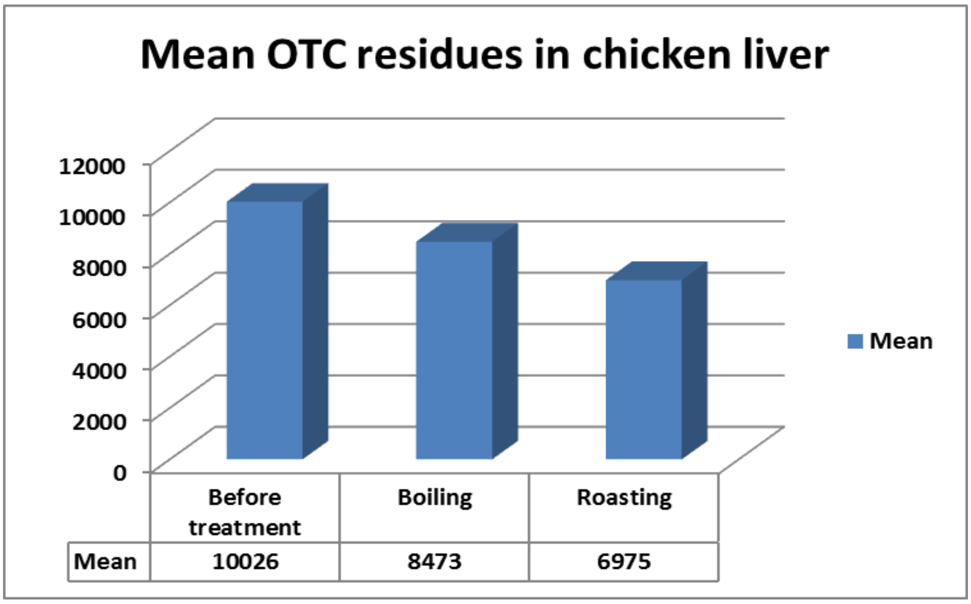

Fig. (3) Mean oxytetracycline residues in examined chicken liver samples

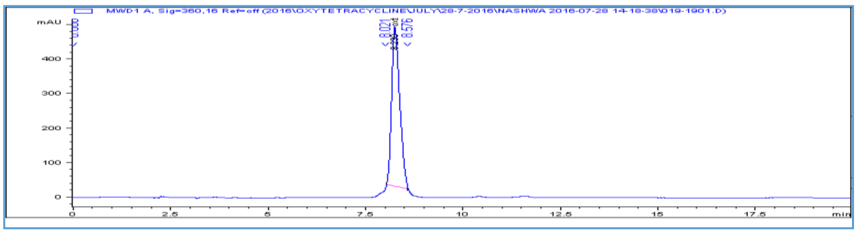

Figure 4. Degradation curves of TC residues in chicken meat during thigh boiling.

Egypt. J. Chem. Environ. Health, 2 (2):598-610 (2016)
On line ISSN: 2536-9164.

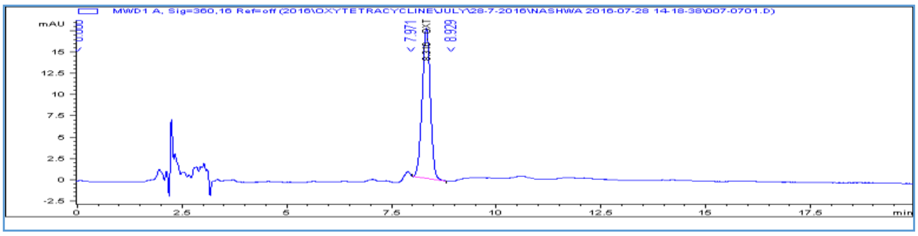

Figure 5. Degradation curves of TC residues in chicken meat during breast boiling.

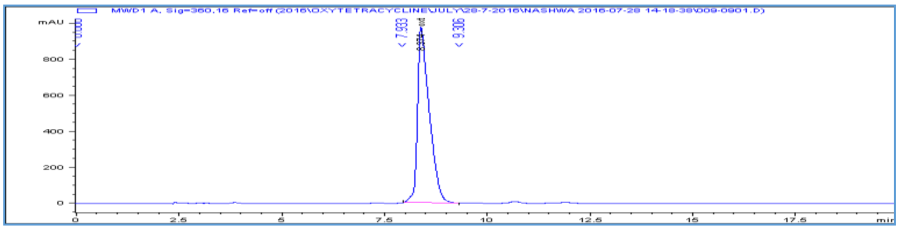

Figure 6. Degradation curves of TC residues in chicken meat during thigh roasting.

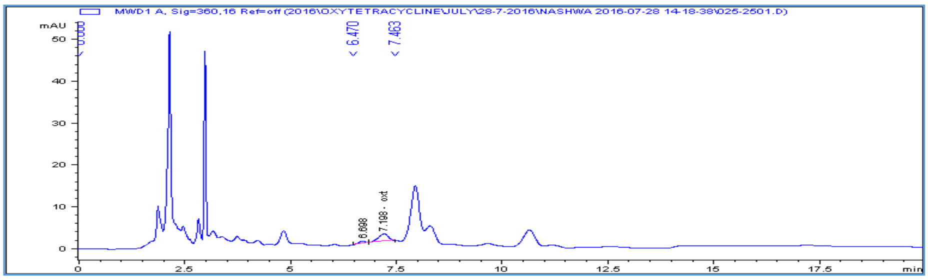

Figure 7. Degradation curves of TC residues in chicken meat during thigh roasting.

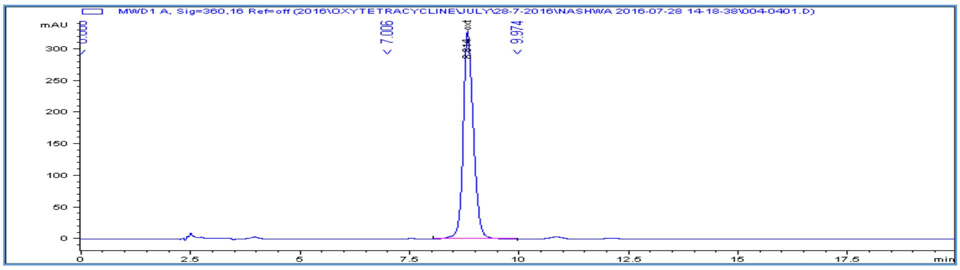

Figure 8 Degradation curves of TC residues in chicken meat during thigh microwave .

Egypt. J. Chem. Environ. Health, 2 (2):598-610 (2016)
On line ISSN: 2536-9164.

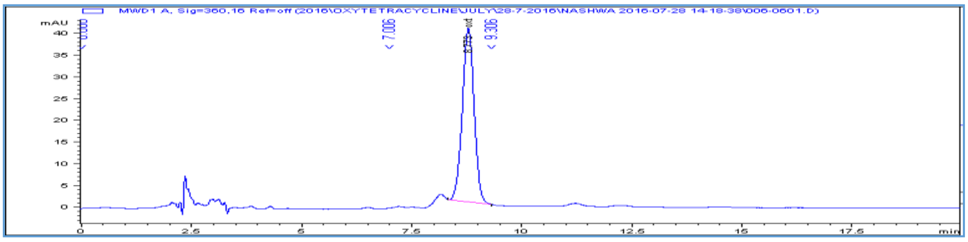

Figure 9 Degradation curves of TC residues in chicken meat during breast microwave.

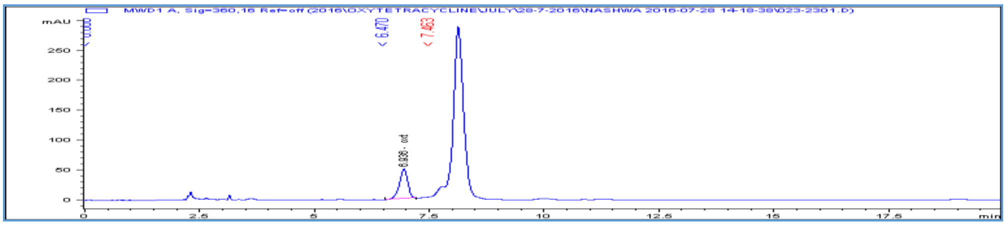

Figure 10 Degradation curves of TC residues in chicken meat during liver boiling.

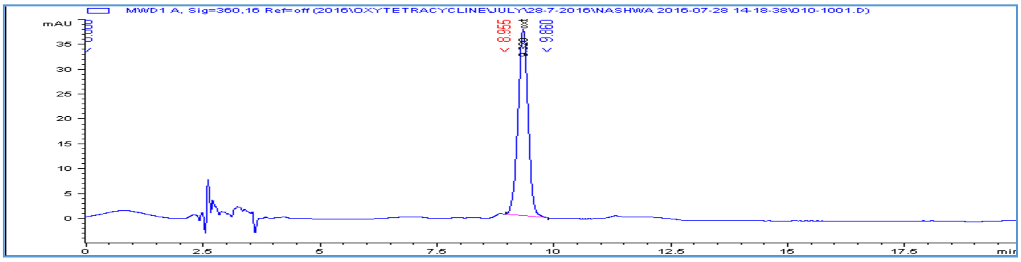

Figure 11. Degradation curves of TC residues in chicken meat during breast roasting.

Conclusions

The present study implies that OTC residues are unstable drugs that will be degraded during cooking, rendering chicken meat apparently safe for human consumption according to the permissible limit cited by the **Commission Regulation (EC) No. 37/2010.** It can be concluded that sufficient cooking temperature and time can have a great effect on OTC residual level and provides margin of safety for consumers. This result shall help the processors and quality control officials to evaluate the presence of these antibiotics in heat-processed products rather than the raw material, also be useful for restaurants and housewives to prepare a safer meal. Education programs and health education should be imposed for Antibiotic residues.

References

Al-Ghamdi, M. S., Al-Mustafa, Z. H., El-Morsy, F.,Al-Faky, A., Haider, I. and Essa, H. (2000) Residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. Public Health 114: 300-304.

Aliu, Y.O (2004): A paper presented on Veterinary Drug Residues in Nigeria s Food at National Awareness Training Programme on Food contaminants and Residues . Women Development Centre, Kaduna, 6-7 may 2004 and NAFDAC Auditorium , Oshodi , Lagos , 20-21 May 2004 .

Booth, N.H. and McDonalid , L.F. (1988): Veterinary pharmacology and therapeutics . 5th Ed . The lowa state Univ . press / Ames, USA .

Butaye , P. ; Devriese, L.A. and Haesebrouck, F .(2001) : Differences in antibiotic resistance patterns of Enterococcus faecalis and Enterococcus faecium strains isolated from farm and pet animals . Antimicrobial Agentss and Chemotherapy . 45: 1347-1378.

Cannavan A. (2004): Capacity of Building for Veterinary Drug residue Monitoring Programmers in Developing Countries . Joint FAO / WHO workshop on Residues of Veterinary Drug without ADL/ MRL – Bangkok .

European Commission. (2010). On pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.Commission Regulation (EC) 37-2010.

Fadwa Fathy M. and Ali M. Ahmed1 and Mohamed K. Moursi(2015) :Effect of Cooking Methods on Antibiotic Residues in Broiler ChickenMeat **2nd Conference of Food Safety, Suez Canal University, Faculty of Veterinary MedicinVolume I August 2015 Page 76-.81**

FAO/WHO. (2004): Residues of some veterinary drugs in animals and foods. Sixty-second report of the JointFAO/WHO Export Committee on Food Additives.

Ibrahim, A. and Moats, W. A. (1994) Effect of cooking procedures on oxytetracycline residues in lamb muscle. J. Agric. Food Chem. 42: 2561-2563.

Javadi, A. (2011) Effect of roasting, boiling and microwaving cooking method on doxycycline residues in edible tissues of poultry by microbial method. Afr. J.Pharm. Pharmacol. 5: 1034-1037.

Kan, C.A. (1995): Residues probability and their occurrence and efficiency of their detection. Misset – world poultry J. 11(10): 67-73.

Moats, W. A. (1988): Inactivation of antibiotics by heating in foods and other substrates: a review. J. Food Prot. 51:491-497.

Nguyen, V.; Li, M.; Khan, M.; Li C. and Zhou G. (2013): Effect of cooking methods on tetracycline residues in pig meat. African J. of Pharmacy and Pharmacology, 7: 1448- 1454

O´brine, J.J.; ***Campbell, N. and Conghen, T. (1981)***: Effect of Cooking and cold storage on biologically active antibiotic residues in meat. J. Hyg. 87: 51-53.

Ramaswamy, H. S. and Chen, C. R.(2004):. Vegetable preservation and processing. In "Canning Principles". pp.67-90. Hui, Y. H., Ghazala, S., Graham, M. D., Murrell,K. D. and Nip, W. K. eds. Marcel Dekker Publishers,New York, U.S.A.

Salah H. Abou-Raya1, Ali R. Shalaby, Nadia A. Salama1, Wafaa H. Emam2 And Fathy M. Mehaya (2013) : Effect of Ordinary Cooking Procedures on Tetracycline Residues in Chicken Meat Journal of Food and Drug Analysis, Vol. 21, No. 1, 2013, Pages 80-86

Salama, N. A., Abou-Raya, S. H., Shalaby, A. R., Emam, W. H. and Mehaya, F. M. (2011) Incidence of tetracycline residues in chicken meat and liver retailed to consumers.Food Addit. Contam. B 4: 88-93.

Shalaby, A. R., Salama, N. A., Abou-Raya, S. H., Emam, W. H. and ehaya, F. M. 2011. Validation of HPLC method for determination of tetracycline residues in chicken meat and liver. Food Chem. 124: 1660-1666.

Shanker ,B. ,P.; Manjunatha Prabhu, B.H. ; Chandan , S.; Ranjith , D. and Shivakumar ,V .(2010) : Rapid methods for detection of veterinary drug residues in meat veterinary world ,3(5): 241-246 **.**

Silva, F. and Azevedo, C. A.(2006): A New version of the assistant-statistical assistance software. In "World Congress on Computers in Agriculture". pp. 393-396. Anais: American society of agricultural engineers.

Thomas, B.B. (1994): Veterinary drug therapy. "Text Book" 5th Ed Lea and Febiger , Awauerly Company press, USA .

Vásquez-Moreno, L., Bermúdez A, M. C., Languré, A.,Higuera-Ciapara, I., Díaz de Aguayo, M. and Flores, E. (1990): Antibiotic residues and drug resistant bacteria inbeef and chicken tissues. J. Food Sci. 55: 632-634.