Original Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 3 Number 1, January - June 2017
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.3117.2

Effect of Microwaving and Some Cooking Methods on Enrofloxacin in Chicken Meat

R. Gogoi*, D.C. Roy*, L.C. Lahon*, S. Sarma, S.K. Laskar*****

*Dept. of P harmacology and Toxicology, **Dept. of Veterinary Biochemistry, ***Dept. of LPT, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India.

Abstract

The present study was undertaken to determine the effects of microwaving and some cooking methods like boiling and deep-frying on Enrofloxacin (ENR) in chicken meat. Chicken meat samples incurred with known concentration of ENR were subjected to these cooking procedures. The cooked samples were then analyzed to record the level of ENR residue using Ultra High Performance Liquid Chromatography (UHPLC) system. The results showed the reduction in concentration of ENR residue after different cooking processes. The most reduced level of ENR in cooked meat samples was observed in microwaving followed by deep-frying and then boiling. The result shows significant reduction in ENR level in chicken meat after cooking. It may be concluded that cooking of meat leads to decrease in the concentration of ENR.

Keywords: Chicken; Cooking; Enrofloxacin; Microwaving; Residue.

Introduction

Fluroquinolones are a group of synthetic antimicrobial agents that have a wide spectrum of activity and high efficacy against various microbial infections. They act by inhibiting the DNA-gyrase which affects the stability of the DNA configuration of the bacterial DNA molecule during cell division (Xu *et al*., 2006). They are commonly used for the treatment of urinary tract and enteric infections in humans (Salehzadeh *et al*., 2007). These agents are normally used for treatment and prevention of infectious disease in farm animals (Maraschiello *et al*., 2001; Dipeolu *et al*., 2002). They are also used as growth promoters (Okerman *et al*., 1998). Antibiotic residues in food can cause hazardous effects to human health. Allergic reactions, imbalance of intestinal microflora, bacterial resistance to antibiotics are some of the adverse effects (Cunha, 2001; Kirbis, 2006).

Enrofloxacin (ENR) is a synthetic fluoroquinolone antimicrobial agent which is administered orally to poultry for the treatment of infections of the respiratory and alimentary tract (Posyniak *et al*., 2001). Levels of drug residues in raw meat and animals products is regulated. Codex Alimentarius Commission (2012) have established maximum residue limit (MRL) of 0.1 µg/g for ENR in meat. Since most foods of animal origin are cooked before consumption, ENR levels in the tissue are dependent on the type of cooking (Lolo *et al*., 2006). Thus the present study was undertaken to see the cooking effects on ENR level in cooked meat.

Materials and Methods

Preparation of Samples

About 100 g of chicken meat sample free from residue was taken and minced and fortified with 1.0

Reprint Request: Rajkishor Gogoi, Department of Pharmacology & Toxicology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam.
E-mail: rajvety@rediffmail.com

(Received on 22.12.2016**, Accepted on** 09.01.2017**)**

µg/g of known standard. The mixtures were then made into portions of 20g chicken meat balls.

Cooking Procedure

Cooking procedures such as boiling, deep-frying and microwaving was performed to study the effects. The chicken meat balls were boiled at 100° C for 5 and 10 mins respectively. In case of deep-frying, the chicken meat balls were fried in a pan with sunflower cooking oil at 170° C for 3 and 6 mins respectively. In case of Microwaving, the Chicken meat balls were placed at the turntable of a microwave oven. The samples were cooked under full power (800 W) for 1 and 2 mins respectively. The temperature during cooking was 100°C. Samples were then processed and analyzed using UHPLC to record the level of residue.

Extraction and Clean Up

HPLC grade water was added to the cooked sample and then homogenized. About 5 g of the sample was transferred to a glass test tube and added 2 ml of 0.1 M phosphate buffer (pH 7.2) and then mixed. After adding 10 ml of dichloromethane, the mixture was sonicated in an ultrasonicator. The sonicated sample was then left undisturbed for 15 mins for allowing the extract to dissolve in the solvent. The sample was then centrifuged at 10,000 rpm for 15 mins at 0° centigrade in a refrigerated centrifuge machine. The supernatant was separated and filtered through a Whatman filter paper No. 42.

Cleanup of the extract was done by using Solid Phase Extraction (SPE) method. The filtrate was loaded on a C_{18} polymeric cartridge preconditioned with 2.5 ml of methanol and 2.5 ml of HPLC grade water. The cartridge containing the sample was washed with 3 ml of water and then finally eluted with 3 ml of methanol.

The extract so obtained was filtered through a syring filter (0.2µm). Finally, 20µl of the eluted sample was then injected into the UHPLC system for analysis.

Chromatographic condition

A mobile phase of Water: Acetonitrile (70:30 v/v) was used. The flow rate was kept at 1.0 ml/min keeping mode as isocratic. The wavelength for the detector was set at 277 nm.

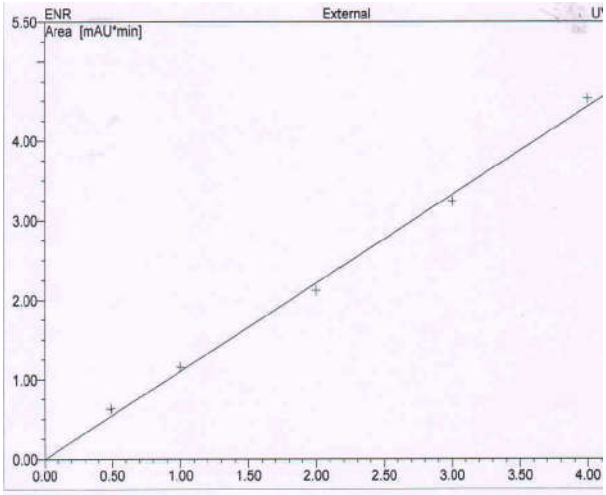
Quantification

About 10 mg of pure Enrofloxacin standard was dissolved in 100 ml of HPLC grade water with drop of HCl until complete dissolution to obtain a concentration of 100µg/ml. Further dilutions were made from this solution in the descending concentration of 4.0, 3.0, 2.0, 1.0 and 0.5µg/ml respectively. An aliquot of 20µl each of these solutions were injected into the UHPLC system. Peak areas were recorded. A standard calibration curve with coefficient of determination of 99.55 % was obtained by plotting concentration of standard solutions against peak areas obtained (Figure 1).

Results and Discussion

Microbiological methods are the preliminary screening methods for detection of antibiotic residues in food of animal origin (Hussein, 2004). Screening methods allow preliminary detection of a wide spectrum of antibiotics (Haasnoot *et al.*, 1999) but they cannot be used for quantitative analysis for antibiotic residues. A positive result should be confirmed with more precision methods like chromatographic technique (Ferrini *et al.*, 2006). Thus, the present study was performed with Ultra High Performance Liquid Chromatography (UHLPC).

The present method revealed that calibration curves showed good linearity (r^2) of 0.996 over the range of 0.5 to 4.0 µg/ml. Accuracy and recovery was in the range of 92-99% in chicken meat indicating that the method was a validated method.



No.	Ret.Time min	Peak Name	Cal.Type	Points	Coeff.Det. %	Offset	Slope
1	2.28	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	3.20	ENR	Lin	4	99.5570	0.0000	1.1077
Average:					99.5570	0.0000	1.1077

Fig. 1: Standard calibration curve of Enrofloxacin (ENR)

Table 1: Effect of Cooking on ENR level in Chicken meat

Cooking Process	Boiling		Deep Frying		Microwaving	
Time (mins)	5	10	3	6	1	2
ENR Concentration (µg/g)	0.845± 0.021	0.567± 0.020	0.848 ± 0.019	0.468 ± 0.019	0.668± 0.015	0.291 ± 0.027

Lolo *et al*., 2006 reported that when the chicken samples were boiled at 100°C for 10 min and microwaved at 800 W for 3.5 min there was a reduction in concentration.The results of boiling and microwaving in this research confirm the findings of our study about the decrease of ENR activity after cooking. As shown in Table 1, after 1 min of microwaving, ENR level in the samples was found to be 0.668±0.015 µg/g. The samples which were micro waved for 2 mins showed further decrease in the level of ENR which was 0.291 ± 0.027µg/g.

Hence ENR level reduced significantly by 33.2 % and 70.9 % after 1 min and 2 min of microwaving. ENR level in the samples after 5 mins and 10 mins of boiling was found to be 0.845±0.021 µg/g and 0.567±0.020 µg/g respectively. ENR residues reduced significantly by 15.5% after 5 mins of boiling while after 10 mins it further reduced by 43.3%. Similarly, ENR level reduced significantly by 15.2% and 53.2% after 3 mins and 6 mins of deep frying. After 3 mins of deep-frying ENR level in the samples were found to be 0.848 ± 0.019 µg/g. The samples which were deep-fried for 6 mins showed further decrease in the level of ENR to 0.468 ± 0.019 µg/g. It also corroborated well with the findings of Van Egmond *et al*., 2000 where it was reported that Enrofloxacin residue in pork reduced to 68% after cooking.

Conclusion

It can be concluded from the present study that cooking processes can cause a significant decrease in the level of ENR in meat. It was also found that cooking time and temperature played a major role in reducing the level of ENR. Microwaving can be regarded as the best cooking process followed by deep-frying and then boiling.

Acknowledgement

The authors are grateful to ICAR and AAU, Jorhat for the help during this research.

References

1. Codex Alimentarius Commission. Maximum Residue Limits for Veterinary Drugs in Food. FAO/ WHO Rome. 2012.
2. Cunha B.A. Antibiotic side effects. Med. Clin. North. Am., 2001; 85(1):149-185.
3. Dipeolu M.A., Alonge D.O. Residue of streptomycin in meat sold for human consumption in some states of S.W. Nigeria. Arch. Zootec. 2002; 51: 477-480.
4. Ferrini A.M., Mannoni V., Aureli P. Combined plate microbial assay (CPMA): A 6 plate method for simultaneous first and second level screening of antibacterial residues in meat. F. Add. Cont. Part A., 2006; 23(1):16-24.
5. Haasnoot W., Stouten P., Cazemier G., Lommen A., Nouws F.M., Keukens H.J. Immunochemical detection of aminoglycosides in milk and kidney. Anal. 1999; 124:301-305.
6. Hussein K. Experimental design for the microbiological four-plate test for the detection of sulphadimidine residues at the levels of concern. Bull. Vet. Inst. Pulawy, 2004; 48(4):403-407.
7. Kirbis A. Microbilogical 5-plate screening method for detection of tetracyclines, aminoglycosides, cephalosporines and macrolides in milk. Slo. Vet. Res. 2006; 43(4):161-168.
8. Lolo M., Pedreira S., Miranda J.M. , Vazquez B.I., Franco C.M., Cepeda A., Fente C. Effect of cooking on enrofloxacin residues in chicken tissue. F. Add. Cont. Part A., 2006; 23(10):988-993.
9. Maraschiello C., Cusido E., Abellan M., Vilageliu J. Validation of an analytical procedure for the determination of the fluoroquinolone enrofloxacin in chicken tissues. J. Chromatogr. B, 2001; 754(2):311-318.
10. Okerman L., Van Hoof J., Debeuckelaere W. Evaluation of the european four-plate test as a tool for screening antibiotic residues in meat samples from retail outlets. J. Assoc. off. Anal. Chem., 1998; 81:51-56
11. Posyniak A., Zmudzki J., Semeniuk S. Effects of the matrix and sample preparation on the determination of fluoroquinolone residues in animal tissues. J. Chrom. A., 2001; 914(1-2):89-94.
12. Salehzadeh F., Salehzadeh A., Rokni N., Madani R.,

Golchinefar F. Enrofloxacin residue in chicken tissues from Tehran slaughterhouses in Iran. Pak. J. Nutr., 2007; 6(4):409-413.

13. Van Egmond H.J., Nouws J.F.M., Schilt R., Van Lankveld-Driessen W.D.M., Streutjens-van N.E.P.M., Simons F.G.H. Stability of antibiotics in meat during a stimulated high temperature destruction process. Proceedings of the Euro residue conference IV, Veldhoven, Netherlands, 2000.p.430-438.
14. Xu W., Zhu X., Wang X., Deng L., Zhang G. Residues of enrofloxacin, furazolidone and their metabolites in Nile tilapia(Oreochromis niloticus). Aqua., 2006; 254:1–8.