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Effect of cooking on ciprofloxacin level in chicken meat

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

Keywords: Chicken, cooking, ciprofloxacin, microwaving

Introduction
Fluoroquinolones 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) [14]. They are commonly used for the treatment of urinary tract and enteric infections in humans (Salehzadeh *et al*., 2007) [12]. These agents are normally used for treatment and prevention of infectious disease in farm animals (Maraschiello *et al.*, 2001; Dipeolu *et al*., 2002) [9, 3]. They are also used as growth promoters (Okerman *et al*., 1998) [10]. 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) [2, 7].
Ciprofloxacin (CPR) 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) [11]. 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 CPR in meat. Since most foods of animal origin are cooked before consumption, CPR levels in the tissue are dependent on the type of cooking (Lolo *et al*., 2006) [8]. Thus the present study was undertaken to see the cooking effects on Ciprofloxacin 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 µg/g of known standard. The mixtures were then made into portions of 20 g 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 100º 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 170º 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 100º 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.

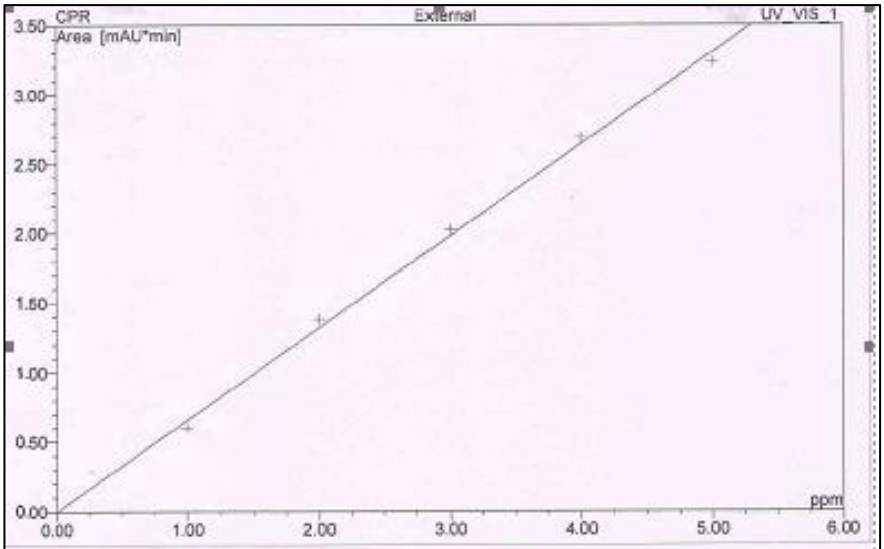
The extract so obtained was filtered through a syringe filter (0.2µm). Finally, 20µl of the eluted sample was then injected into the UHPLC system for analysis.

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 Ciprofloxacin 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 5.0, 4.0, 3.0, 2.0 and 1.0 µg/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 99.63 % was obtained by plotting concentration of standard solutions against peak areas obtained (Figure 1).



No.	Ret.Time min	Peak Name	Cal.Type	Points	Coeff.Det. %	Offset	Slope	Curve
1	3.20	CPR	Lin	5	99.6352	0.0000	0.6602	0.0000
2	6.11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:					99.6352	0.0000	0.6602	0.0000

Fig 1: Standard calibration curve of Ciprofloxacin (CPR)

Results and Discussion

Microbiological methods are the preliminary screening methods for detection of antibiotic residues in food of animal origin (Hussein, 2004) [6]. Screening methods allow preliminary detection of a wide spectrum of antibiotics (Haasnoot *et al*., 1999) [5] 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) [4]. 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 1.0 to 5.0 µg/ml. Accuracy and recovery was in the range of 92-99% in chicken meat indicating that the method was a validated method.

Lolo *et al*., 2006 [8] 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 CPR activity after cooking. As shown in Table 1, after 1 min of microwaving, CPR level in the samples was found to be 0.680±0.014 µg/g. The samples which were micro waved for 2 mins showed further decrease in the level of ENR which was 0.264 ± 0.020µg/g. Hence CPR level reduced significantly by 33.2 % and 70.9 % after 1 min and 2 min of microwaving. CPR level in the samples after 5 mins and 10 mins of boiling was found to be 0.871±0.020 µg/g and 0.597±0.015 µg/g respectively. CPR residues reduced significantly by 15.5 % after 5 mins of boiling while after 10 mins it further reduced by 43.3 %. Similarly, CPR level reduced significantly by 15.2 % and 53.2 % after 3 mins and 6 mins of deep frying. After 3 mins of deep-frying CPR level in the samples were found to be 0.868 ± 0.021 µg/g. The samples which were deep-fried for 6 mins showed further decrease in the level of CPR to 0.570 ± 0.018 µg/g. It also corroborated well with the findings of Van Egmond *et al*., 2000 [13] where it was reported that Ciprofloxacin residue in pork reduced to 68% after cooking.

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

Cooking Process	Boiling		Deep Frying		Microwaving	
Time (mins)	5	10	3	6	1	2
CPR Concentration (µg/g)	0.871±0.020	0.597±0.015	0.868 ± 0.021	0.570 ± 0.018	0.680 ± 0.014	0.264 ± 0.020

n=6

Conclusion

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

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References

1. Codex Alimentarius Commission. Maximum Residue Limits for Veterinary Drugs in Food. FAO/WHO Rome, 2012.
2. Cunha BA. Antibiotic side effects. Med. Clin. North. Am. 2001; 85(1):149-185.
3. Dipeolu MA, Alonge DO. Residue of streptomycin in meat sold for human consumption in some states of S.W. Nigeria. Arch. Zootec. 2002; 51:477-480.
4. Ferrini AM, 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 FM, Keukens HJ. 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 JM, Vazquez BI, Franco CM, Cepeda A *et al*. 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 HJ, Nouws JFM, Schilt R, Van Lankveld-Driessen WDM, Streutjens-van NEPM, Simons FGH. Stability of antibiotics in meat during a stimulated high temperature destruction process. Proceedings of the Euro residue conference IV, Veldhoven, Netherlands, 2000, 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.